THE SYNTHESIS AND PHYSICAL PROPERTIES OF SELECTED TRIACYLGLYCEROLS

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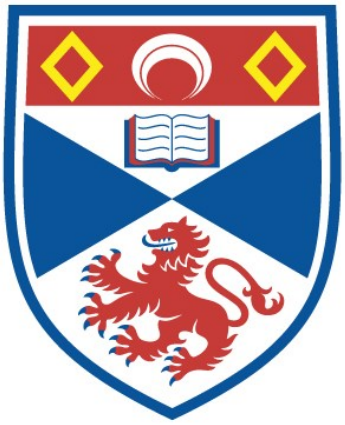
A Thesis Submitted for the Degree of PhD
at the
University of St Andrews



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A Thesis

presented for the degree of

DOCTOR OF PHILOSOPHY

in the Faculty of Science of the

University of St. Andrews

by

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March 1985

United College of St. Salvator
and St. Leonard, St. Andrews.

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To My Family

D E C L A R A T I O N

I declare that this thesis is my own composition, that the work of which it is a record has been carried out by me, and that it has not been submitted in any previous application for a Higher Degree.

This thesis describes the results of research carried out at the Department of Chemistry, United College of St. Salvator and St. Leonard, University of St. Andrews and at the Cadbury Schweppes Lord Zuckerman Research Centre, Whiteknights, Reading, under the supervision of Professor F.D. Gunstone since the 1st October 1981.

C E R T I F I C A T E

I hereby certify that David Griffiths has spent twelve terms of research under my supervision, has fulfilled the conditions of Ordinance No. 12 and Resolution of the University Court, 1967, No. 1, and is qualified to submit the accompanying thesis in application for the degree of Doctor of Philosophy.

Professor F.D. Gunstone

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I would like to thank Professor F.D. Gunstone for his help and understanding throughout the course of this work. I am also grateful to Dr. G.G. Jewell, my industrial supervisor, for his assistance during the project and useful discussions concerning Part II of this thesis.

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ABSTRACT

The study of triacylglycerol interactions in the solid phase and their behaviour during crystallisation requires the availability of individual triacylglycerols of proven positional and structural purity. The aim of this work was to prepare some specific triacylglycerols and to examine their crystallisation behaviour as individual samples and in binary mixtures with each other.

The first part of this thesis details the synthesis of glycerol 1-stearate 2-caprate 3-palmitate, glycerol 1-stearate 2-oleate 3-palmitate, glycerol 1-stearate 2-elaidate 3-palmitate, glycerol 1-stearate 2,3-dimyristate and glycerol 1,3-dipalmitate 2-laurate. The unsaturated acids used were isolated from olive oil and modified where required. Methods of analysis for quantitative purity determination are given.

The second part of the thesis involves the examination of the selected triacylglycerols by methods of X-ray powder diffraction and differential scanning calorimetry. The triacylglycerols prepared as described were supplemented by lipid samples from other sources [glycerol 1-stearate 2-laurate 3-myristate, glycerol 1-stearate 2-myristate 3-laurate, glycerol 1-palmitate 2-stearate 3-oleate, glycerol 1-palmitate 2,3-dielaidate, glycerol 1,2,3-triarachidate, glycerol 1,2,3-triplamitate, glycerol 1,2,3-tristearate and cocoa butter]. The polymorphic forms of samples prepared from

solvent and from thermal conditioning were obtained. Five pairs of the triacylglycerols were chosen for examination as binary mixtures. The preparation methods and results are detailed and summarised in temperature vs. composition (phase) diagrams.

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C H A P T E R 1

GENERAL INTRODUCTION

Triacylglycerols are esters of the trihydric alcohol glycerol in which all three hydroxyl groups have been esterified, usually with long-chain carboxylic acids (fatty acids). A mono-acid triacylglycerol contains only one type of acid attached to the glycerol backbone (e.g. tristearin or glycerol 1,2,3-tristearate, Fig. 1):

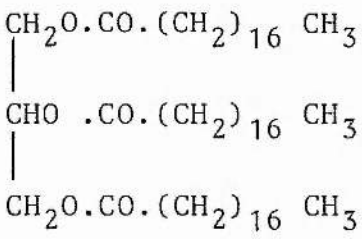


Figure 1

Natural oils and fats are usually mixtures of triacylglycerols each containing two or three different acyl groups. Mono-acid triacylglycerols occur only rarely in nature.

Glycerol 1-stearate 2-caprate 3-palmitate (Fig. 2) is an example of a tri-acid triacylglycerol:

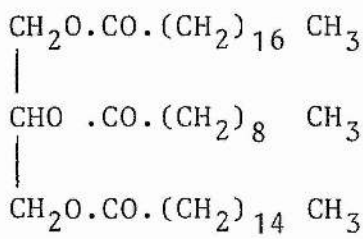


Figure 2

The positions of the acyl groups attached to the glycerol are denoted as 1, 2 or 3. If, as in this case, the acyl groups in the 1 and 3 positions are different then the molecule contains a chiral centre at the C-2 position of the glycerol backbone.

In many natural fats and most synthetic preparations there is a mixture of enantiomers present. Most work is, therefore, done on racemic mixtures and this will be the case throughout this project. Synthetic methods of preparing enantiomeric triacylglycerols are known but are not detailed here.

To abbreviate the nomenclature of the triacylglycerols in this study the first letters of the constituent fatty acid chains are used. For example, the triacylglycerol (Fig. 2) is abbreviated to SCP. The compound prepared is a racemic mixture and could be equally well represented as PCS (see App. 1).

It can be seen from the nature of the general triacylglycerol structure that, even allowing for an equivalence between $R^1R^2R^3$ and $R^3R^2R^1$, there is available a large number of compounds from a fairly small number of fatty acids. Ignoring stereoisomers, a fat containing N different fatty acids may contain $0.5\ N(N+1)$ different triacylglycerols. Thus even with a simple fatty acid composition, natural fats are generally complex mixtures of many triacylglycerols.

The physical properties of triacylglycerols both individually and as mixtures are of importance in industry and in the study of cell metabolism and in membrane structure.

Although the principal biological function of triacylglycerols is thought to be as an energy store, a detailed understanding of their physico-chemical properties can assist in determining the role of related compounds such as phospholipids which generally serve as structural lipids in biological systems.

The physical properties exhibited by triacylglycerols, individually or in a mixture, are determined by the nature and distribution of the fatty acid groups in the molecules. A comprehensive understanding of the properties of naturally produced triacylglycerol systems, therefore, calls for a study of individual triacylglycerol systems and simple mixtures. For this reason it is necessary to prepare pure triacylglycerols of proven structure and then to study some of their physical characteristics. This thesis describes the preparation and analysis of five such triacylglycerols and gives an account of some of their physical properties.

P A R T I

SYNTHESIS

CHAPTER 2

INTRODUCTION TO TRIACYLGLYCEROL SYNTHESIS

The study of triacylglycerol interactions in the solid phase and their behaviour during crystallisation transformations requires the availability of individual triacylglycerols of proven positional and structural purity. Samples of such triacylglycerols from commercial sources, when available, are prohibitively expensive for studies requiring more than fractions of a gram. The preparation of relatively large (several gram) batches of triacylglycerols and an assurance of purity is, therefore, a necessary precursor to triacylglycerol characterisation and the study of compatibility and crystallisation behaviour of triacylglycerol mixtures.

The preparation of individual triacylglycerols presents several problems, as does the selection of the triacylglycerols to be studied. The triacylglycerols selected, and the reasons for their selection, are given later (Part II, Introduction). The choice of synthetic route and the methods of purity analysis are discussed below.

Methods used previously for the synthesis of triacylglycerols and similar compounds[1-3] provide a variety of routes to yield the final products. The route chosen depends largely on the type of

triacylglycerol required i.e. whether mono-, di- or tri-acid and whether any positional symmetry exists which might allow simplification of the route by reducing the number of steps necessary. The triacylglycerols prepared for this study were POS, PES, PCS, SMM and PLP. (Appendix 1)

The triacid triacylglycerols require at least three steps in their preparation in order to assure positional purity of the acyl chains on the glycerol back-bone i.e. it is necessary to isolate mono-, di- and finally tri-acylglycerols or their derivatives. The procedure used[4] was to prepare glycerol 1-stearate followed by glycerol 1-stearate 3-palmitate. This could then be acylated with the appropriate fatty acyl group to yield the required triacylglycerol. The incorporation of the 18.0 acyl chain at the monoacylglycerol stage was recommended and was prepared via the isopropy-lidene glycerol. This afforded a blocking group to minimise acylation of the central carbon of the glycerol and further acylation. Removal of this blocking group could also be performed under conditions which reduced the chance of acyl migration which could cause the 2-isomer to be formed.

Acylation of the glycerol 1-stearate with palmitoyl chloride preferentially occurs at the 3 position of the monoacyl glycerol to yield mainly the 1,3-diacylglycerol. The formation of 1,2-isomer and/or triacylglycerol (SPP) can be monitored by t.l.c. Such byproducts can be removed

by crystallisation with appropriate solvent systems. In some cases it has been reported possible to isolate any triacylglycerol formed at this stage as a useful product of the synthesis [4]. Further acylation by reaction of the diacylglycerol with the required acid chloride and purification by crystallisation was the preferred method of isolating the three tri-acid triacylglycerols.

The preparation of SMM again suits itself to the initial synthesis of glycerol 1-stearate, however, there is no necessity for isolating the diacylglycerol. Acylation of the glycerol 1-stearate with 2.5 molar amount of myristoyl chloride was the route used in this case.

Glycerol 1,3-dipalmitate 2-laurate possesses a useful degree of symmetry which afforded the opportunity to avoid the mono-, di- and finally tri-acylglycerol synthesis route starting from glycerol. By using dihydroxy-acetone as a starting material in place of the glycerol, the two terminal (1- and 3-positions) acyl groups could be attached as the first stage of the synthesis. Reaction of dihydroxy acetone with 2 x molar amount acid chloride followed by reduction of the ketone group with sodium borohydride was the method chosen for preparation of glycerol 1,3 dipalmitate[15]. On isolation of this diacylglycerol, further acylation by acid chloride (lauroyl chloride) yields the desired triacylglycerol PLP.

CHAPTER 3

GENERAL METHODS OF SYNTHESIS AND ANALYSIS

3.1 Preparation of Methyl Esters and TMS Derivatives

3.1.1 Esters

Esterification of free fatty acids and trans-esterification of triacylglycerols was effected by acid catalysed methylation. A sample of lipid (approximately 50 mg) was refluxed with acidic methanol (2% sulphuric acid in methanol, 2 ml) for 30 mins (free acids) or 2 hours (triacylglycerols). Alternatively, the reaction mixture was left at room temperature overnight. A saturated brine solution (5 ml) was added to aid separation and the esters taken up in petroleum ether (2x5 ml). The combined extracts were dried (sodium sulphate) and concentrated under a stream of nitrogen.

3.1.2 TMS derivatives

Monoacylglycerols were converted to trimethylsilyl (TMS) ether derivatives for analysis by gas chromatography. These derivatives provide a series of volatile compounds which can be separated on the gc column at around 200°C with reasonably short retention times without decomposition. The mono-acylglycerol (10 mg) was placed in a test-tube and pyridine (0.5 ml), hexamethyldisilazane (0.15 ml) and trimethyl-chlorosilane (0.05 ml) added. The mixture was shaken and allowed to stand for 10 minutes. Petroleum ether (5 ml)

was added, the sample washed with water (2x1 ml) and dried (sodium sulphate). The petrol solution was concentrated and stored at 5°C if not used immediately.

3.2 Analysis of Methyl Esters and bis-TMS Ethers by Gas Chromatography

A Pye Unicam PU4500 chromatography with flame ionisation detector was used in conjunction with a LDC model 308 computing integrator and a chart recorder. Glass columns (2 m) were packed with SP2300 on chromsorb W, the column being purged with nitrogen and conditioned at approximately 210°C overnight before initial use. The column efficiency, checked with standard esters, had a value of at least 1000 theoretical plates. (Column efficiency = $16(Rt/w)^2$ where Rt = retention time and w = peak width).

The carrier gas was oxygen-free nitrogen at a flow of 60 ml/min. The usual operating temperature depended on the sample chain length but was generally around 190°C for methyl esters and 245°C for TMS derivatives of monoacyl-glycerols. The samples were made up in petroleum ether (redistilled) and a maximum volume of 5 µl injected at a time. If samples required an attenuation scale $<10x10^2$ for a reasonable peak height on the chart recorder, they were concentrated under a stream of dry nitrogen and re-injected. Methyl esters analysed on the SP2300 column were separated on the basis of their chain length and degree of unsaturation. The bis-TMS derivatives of

monoacylglycerols were separated by virtue of the acyl group position on the glycerol backbone, i.e. the positional isomers had different retention times, the 1-monoacylglycerol being eluted later than the 2-monoacylglycerol isomer. The acyl chain length would also effect the retention time of the derivative.

3.3 Gas Chromatography of Triacylglycerols

Triacylglycerols can be separated by temperature programmed gas chromatography according to their gross molecular weights. The separation is dependent on the number of carbon atoms in the molecule and so the triacylglycerols are referred to by the total number of carbon atoms in the three acyl chains. The three glycerol carbon atoms are not included in this value, e.g. glycerol tristearate is termed C54 as is any other triacylglycerol having chain lengths totalling 54 carbons. The separation using this technique does not distinguish between positional isomers, stereoisomers or the level of unsaturation. Triacylglycerols with equal numbers of carbon atoms therefore elute with the same retention time.

A Pye series 104 chromatograph with flame ionisation detector was used in conjunction with an oven temperature programme controller and heated injection port. The column used was a short (45 cm) 4 mm ID glass column packed with 3% OV1 on 100/120 mesh Gas Chrom Q. Before packing, the column was washed with toluene, Sylon and methanol to

block any free -OH groups on the glass surface which would otherwise affect the separation. The column was dried, packed and then plugged with silanised glass wool. The heated injection port was fitted with "ultra sep-H" septa as they provided a low bleed at the relatively high temperatures necessary for triacylglycerol separation.

To obtain maximum column efficiency, a carefully controlled and time consuming conditioning procedure was required. With correct conditioning of the column and the use of appropriate accessories, such as the high temperature septa, the technique can provide base-line resolution of the triacylglycerols of coconut oil and minimal base-line drift without the use of a reference column (Appendix 2). The conditioning procedure used was based on that given by Hammond (1981, (5)). Nitrogen (oxygen free) was passed through the column at a flow rate of 60 ml/min for 5 mins at room temperature. The temperature was then raised to 50°C for 10 mins. The injection port heater was switched on to 360°C, the nitrogen flow rate reduced to 5 ml/min and the oven temperature programmed from 50°C to 370°C at 1 C/min. After 36 hours at 370°C the column was cooled to 250°C and the nitrogen flow increased to 60 ml/min. A solution of coconut oil in chloroform (10% w/v) was injected (1 µl) and the oven temperature increased to 360°C at 4 C/min. The injection port was maintained at 360°C throughout. The column was left at 360°C for 12 hours and then cooled to 250°C. A further sample of the coconut oil solution was injected and the temperature programme repeated.

This procedure was repeated until base-line resolution of the coconut oil triacylglycerols was obtained and the column bleed was minimal. On occasion, the required column efficiency was not obtained after 5 such injections and so the column was discarded and a fresh column prepared and conditioned (see Appendix 2).

Triacylglycerol samples were made up in chloroform and 1 µl injected at 250°C. The oven temperature programme was 250 - 360°C at 4 C/min with a nitrogen flow of 60 ml/min. The injection port temperature was always maintained at 360°C.

3.4 Thin Layer Chromatography

Thin layer chromatography was used both as an analytical and a preparative technique. As an analytical method it provided a general purity check for starting materials and reaction products and its relative speed compared to other analytical methods made it useful for monitoring the progress of certain reactions and purification steps. Preparative tlc was used in the isolation of 2-monoacylglycerols from the other products of triacylglycerol lipolysis.

In all cases plates were prepared by spreading an aqueous slurry of "Kieselgel G" silica (2 ml water/g silica) over glass plates. Certain separations (see below) required additional reagents incorporated into the silica layer. These were dissolved in the water before addition of the silica. This ensured even distribution of the

reagent through the coating. Analytical plates were normally 5x20 cm and the silica coating applied 0.25 mm thick. Preparative plates were normally 20x20cm and were coated 0.5 or 0.75 mm thick depending on the loading required for separation. Plates were left to dry for 10 mins before being activated in an oven at 110°C for at least 2 hours. Plates were allowed to cool after activation and were stored in dry conditions if not used immediately.

Where samples were to include acylglycerols, possible acyl migration during elution was minimised by the incorporation of 5% (w/w) boric acid into the coating. Separation of lipids according to double bond configuration required the addition of 5% (w/w) silver nitrate.

Once a plate had been eluted, it was developed by spraying an appropriate reagent over the surface. If the separation did not require the recovery of the sample, then visualisation was effected by spraying with a solution of phosphomolybdic acid (10%) in ethanol and then charring the lipid material in an oven (100°C). This method was convenient and sensitive enough for the analytical separation. Development of preparative plates necessitates the use of a nondestructive reagent and so a solution of 2',7'-dichlorofluorescein (0.1%) in methanol was used. All bands of lipid could then be made visible by holding the plate under UV light. The required bands were outlined and carefully removed using clean razor-blades. The lipid material was extracted from the silica by washing with solvent ether.

All bands collected from the plate were then analysed by gc without interference from the fluorescein which remained on the gc column. If necessary the fluoroscein could be removed by dissolving the sample in chloroform/methanol (2:1 by volume) and washing with dilute ammonia.

3.5 Lipolysis

Complete methanolysis of a triacylglycerol indicates the presence and relative proportions of individual acyl groups but gives no information about their distribution on the glycerol backbone. The regiospecific lipolysis of the 1 and 3 ester positions of triacylglycerols by pancreatic lipase is the basis of a useful method for determination of positional purity[6].

The experimental conditions for lipolysis were critical for the success of the analysis as may be expected for a reaction involving an enzyme. Factors of importance include temperature, pH, the presence of bile salts, calcium ions and, especially important, vigorous agitation to encourage emulsification. Lipases are water soluble proteins and contact between the enzyme and triacylglycerol occurs only at the interface between the aqueous and oil phase. The conditions were selected to provide a short reaction time and so minimise the possibility of acyl migration which would lead to erroneous results. The reaction was terminated when necessary by the addition of strong acid solution to neutralise the enzyme activity.

On completion of the reaction the 2-monoacylglycerols were isolated by preparative thin layer chromatography. Other products included free fatty acids and di- and triacylglycerols. The 2-monoacylglycerol band was removed from the plate and methyl esters prepared for analysis by gc.

This technique required only about 50 mg of triacylglycerol and the complete analysis need only take a few hours provided the conditions and reagents have been correctly chosen.

Method

Pancreatin was washed with acetone (2x, 1:5 wt/vol) and diethyl ether (2x, 1:5 wt/vol) to remove any fat material from the commercially prepared sample. A bile salt solution of 0.037% sodium taurocholate in ammonia buffer at pH 8.4 was prepared. Triacylglycerol (80 mg) was transferred to a sample tube with hexane (0.4 ml) and bile salt/buffer solution (4 ml). The mixture was shaken vigorously for 5 minutes in a water bath at 41°C. Calcium chloride solution (23% wt/vol, 0.5 ml) and pancreatin (120 mg) were then added. Vigorous shaking for 10 minutes at 41°C was followed by the addition of hydrochloric acid solution (2 ml, 1 M) to stop the reaction. The mixture was extracted with diethyl ether (3 x 10 ml) and the combined extracts washed with water (2 x 5 ml) and dried (anhydrous sodium sulphate).

The 2-monoacylglycerols were separated from unreacted material and biproducts using preparative thin layer chromatography. Silica gel plates (20 x 20 cm, 0.5 mm thick) were developed with petroleum ether, diethyl ether, formic acid (80:20:1) and the lipid material made visible by spraying with 2,7-dichlorofluorescein in methanol (0.1%). The 2-monoacylglycerols were scraped off the plate and 2% sulphuric acid in methanol (5 ml) added, directly. On leaving at room temperature overnight, the mixture was extracted with petrol (3 x 5 ml), washed with water and dried (anhydrous sodium sulphate). The methyl esters were then analysed by gas chromatography.

3.6 Urea Crystallisation

The crystallisation of urea from a methanol solution containing long chain molecules can result in the formation of inclusion compounds. The introduction of a *cis* double bond in a long chain molecule produces a kink in the normally straight chain. The larger the number of such bonds, the greater the effect on the shape of the molecule. As urea adducts preferentially contain straight chain compounds, this presents a method for separation of fatty acids by virtue of their *cis* unsaturation. By using the correct crystallisation conditions polyene, monoene and saturated acids can be separated from one another. During the purification of oleic acid, several urea crystallisations were used to remove saturated acids (16:0, 18:0) from the olive acid mixture.

Urea was dissolved in warm methanol (2 ml/g) and the acids added. The amount of urea required for the weight of acids to be removed was determined using the information given in Appendix 2, (Table 2[7]). The solution was allowed to crystallise overnight at 0°C before the precipitate was filtered off and pressed firmly to remove the mother liquor. Cold methanol saturated with urea was used to wash the precipitate. The methanol was removed from the mother liquor (RFE) and the resulting crystals dissolved in the minimum of water. The solution was acidified with hydrochloric acid (3 M) and extracted twice with petrol. The organic phase was washed with water and dried (sodium sulphate). On removal of the solvent (RFE) the fatty acids were recovered, weighed and analysed by gas chromatography of their methyl esters. A sample of the product was also examined by tlc for oxidation products and methyl ester formation (from contact with methanol) if left in storage for a prolonged period and to ensure handling conditions were not too severe. If excess urea was used or if the crystallisation conditions were not correct, a significant amount of the desired unsaturated material could have also formed adducts and have been precipitated. The urea precipitate was therefore kept until the analysis of the mother liquor was complete.

3.7 Low Temperature Crystallisation

The purification of oleic acid from olive oil required the reduction of the polyunsaturated acid level (mainly 18:2, linoleic acid) by low temperature crystallisation[7] This involved the olive acid mixture being dissolved in petroleum ether and the gradual lowering of the solution temperature until the oleic acid crystallised, leaving the bulk of the polyunsaturated material in the mother liquor. The low temperatures required (-40°C) to crystallise the oleic acid from solution were obtained using an acetone/solid carbon dioxide bath with insulation sufficient to allow a constant crystallising temperature over several hours with minimum supervision. The desired temperature was reached slowly by the addition of lumps of solid carbon dioxide to the acetone bath over approximately two hours. The crystallisation was left to proceed for a further five hours at constant temperature. The oleic acid crystallised as a white powdery solid which was filtered through a Buchner funnel surrounded by a cooling jacket containing crushed solid carbon dioxide. Cold petrol was passed through the filter prior to the sample. The precipitate was pressed firmly with a cold beaker containing crushed solid carbon dioxide to remove the mother liquor and then washed with cold petrol. On standing, the oleic acid melted and was stored in a refrigerator until required. The mother liquor was dried (sodium sulphate) and the petrol removed (RFE). The methyl

esters of the oleic acid precipitate and of the mother liquor were analysed by gc. If the precipitate contained > 1.0% polyunsaturates, then a further low temperature crystallisation was required at a slightly higher temperature. A second crop of precipitate was obtained from the mother liquor if the oleate concentration was sufficiently high (> 85%).

3.8 Preparation of Acid Chlorides

Fatty acid chlorides were required for the preparation of di- and tri-acylglycerols. The saturated acids (16.0, 14.0, 12.0 acids) were reacted with thionyl chloride (1.4 molar excess i.e. 2.4 molar amount) for five days at room temperature. To avoid side reactions leading to double bond isomerisation and decomposition, unsaturated acids were reacted with oxalyl chloride (0.8 molar excess). This reaction was also carried out at room temperature but for three days and in the dark. In both cases the reaction flasks were sealed with a calcium chloride drying tube to exclude moisture and to allow the ventilation of hydrogen chloride gas .

On several occasions saturated acid chlorides were prepared successfully in only a few hours by refluxing the reaction mixture in an appropriate solvent, e.g. n-hexane. An attempt to use redistilled petrol (b.pt. 40-50°C) was not successful presumably because the temperature of reflux was not sufficient for the reaction time of a few hours which was used.

The work-up was the same in all cases. Excess of volatile reagents were removed over a steam bath (RFE) during 1.5 hours and the remaining material taken up in petrol. This was washed three times in ice-cold water and immediately dried (magnesium sulphate). The solvent was removed (RFE) to leave the fatty acid chloride which was checked for unreacted acid by infrared spectroscopy. The lack of an "-OH" frequency was noted.

To avoid exposure to moisture and possible hydrolysis of the acid chloride between stages of the work-up, the reaction was effected as quickly as possible and the sample stoppered with a calcium chloride drying tube when being transferred to the rotary evaporator.

CHAPTER 4

SYNTHESIS AND ANALYSIS

4.1 Saturated Acids

The supply of saturated fatty acids stearic, palmitic, myristic, lauric and capric was B.D.H. "specially pure" material as bought from the company. A sample of each batch of material was taken on delivery and esterified in the normal manner for analysis by gas chromatography and thin layer chromatography. Any material showing impurities by gas chromatography totalling > 1% or visible impurities by thin layer chromatography was discarded.

4.2 Preparation of Oleic Acid from Olive Oil

The oleic acid used for triacylglycerol synthesis was isolated from olive oil in a manner similar to that already described [7]. This method involved the hydrolysis of olive oil triacylglycerols to provide a mixture of free fatty acids containing approximately 75% oleic acid. The percentage of oleic acid was increased by a step-wise use of urea crystallisation, low temperature crystallisation and silica column chromatography. The oleic acid content was monitored at each stage by gas chromatography of the methyl esters.

Once the oleic acid had been purified to > 99%, elaidic acid (the trans isomer of oleic acid) was obtained by stereomutation catalysed by nitrous acid [8].

4.2.1 Hydrolysis of olive oil

Olive oil (1.38 kg) was refluxed for one hour with a mixture of potassium hydroxide (250 g), ethanol (800 ml) and water (250 ml). Care was taken not to overheat the mixture. The mixture was cooled in an ice bath and poured into a separating funnel containing crushed ice (1000 ml). This was followed by the addition of 20% sulphuric acid solution (1200 ml). The mixture was shaken thoroughly to ensure neutralisation of all potassium salts. Unsatisfactory mixing at this stage could result in the isolation of soaps during separation. The pH was checked after the expected neutralisation point was reached.

The lower layer was drawn off into another separating funnel and extracted with diethyl ether (400 ml). The ether extract was combined with the initial organic layer and they were washed with water (2 x 200 ml). The organic phase was transferred to a weighed Buchi flask and benzene (400 ml) added for azeotropic removal of the remaining water. The ether and the benzene/water azeotrope were removed (RFE) and heating was continued for at least 30 mins after the solvent flow seemed to have stopped. This provided olive acids (1.27 kg) of composition 16:0 (8.6%), 18:0 (1.9%), 18:1 (76.5%), 18:2 (13.0%).

4.2.2 Urea crystallisation

The first stage in increasing the 18.1 (*cis*) percentage was to remove the bulk of the saturated acids (16.0 and 18.0) by urea crystallisation. During crystallisation the urea adducts preferentially contain straight-chain compounds i.e. the saturated acid components of the mixture. Three urea crystallisations were carried out consecutively on the olive acids. The amount of urea required was calculated using Table 2, Appendix 2.

The percentage of saturated acids to be removed from the olive acid mixture by the initial urea crystallisation was 10.6% and the amount of urea required was therefore:

$$\frac{10.6}{100} \times 1.27 \times 12$$

i.e. 1.62 kg urea.

The urea (1.62 kg) was dissolved in warm methanol (3.3 litres, 2 ml/g urea) and the olive acids (1.27 kg) were added. The solution was allowed to crystallise overnight at 0°C before the precipitate was filtered off and pressed to remove the mother liquor. Cold methanol saturated with urea was used to wash the precipitate.

One tenth of the mother liquor was taken for work-up and analysis. The methanol was removed (RFE) and the resulting crystals dissolved in the minimum of warm water. The solution was acidified with hydrochloric acid (3 M) and

extracted with petroleum ether (2 x 500 ml). The organic phase was washed with water and dried over sodium sulphate. On removal of the petrol 63 g acids were obtained. The total weight of olive acids from the first urea crystallisation was therefore 630 g.

A small sample, converted to methyl esters for analysis by gas chromatography, gave 16:0 (1.2%), 18:0 (trace), 18:1 (82.2%), 18:2 (16.6%).

The saturated acid content had been significantly reduced from 10.6% to 1.2%. After two further urea crystallisations the acids remaining in the liquor (520 g) did not show a significant decrease in the saturated acid content. A peak with a longer retention time was observed which was probably due to the presence of oxidation products formed during prolonged storage between stages and the treatment of the acids during the crystallisation and work-up.

4.2.3 Low temperature crystallisation

The material obtained from the urea crystallisations was still < 90% 18:1 acid and to increase this level significantly, the level of polyunsaturated acids (18.2 and 18.3) was reduced by a series of low temperature crystallisations.

The acids (470 g, 82% oleic acid) were dissolved in petroleum ether (3.75 l, approx. 8 x weight of acids) and cooled slowly to -50°C as described (section 3.5). The

precipitate (180 g, 96.9% oleic acid) contained a late running impurity (1.8%). The mother liquor (290 g, 84.7% oleic acid) was stored at 0°C for future use.

The precipitate was recrystallised similarly three more times, however no significant increase in the oleic acid content was observed. The final precipitate (55 g 96% oleic acid) was stored at 0°C until purified further (4.2.4). The mother liquors from all four crystallisations were combined along with stock acids (95% oleic acid). This provided 256 g acids containing 93.2% oleic acid. This material was subjected to two further low temperature crystallisations and a urea crystallisation which yielded 145 g acids of 95.2% purity.

All samples of acids and their esters were dark yellow in colour and had a late running gas chromatography peak with a retention time approx. 1.5 times that of the oleate ester. This material was not removable by urea or low temperature crystallisation and so the individual batches of oleate concentrate were further purified by silica column chromatography.

4.2.4 Column chromatography

Silica ("Sorbsil-60") column chromatography was used to remove oxidation products and coloured impurities from the acids already upgraded by urea and low temperature crystallisation. The sample weight/silica gel ratio used was generally around 1:20 and the product was collected with approximately 70% yield.

The columns were made up in petroleum ether and the acids added to the top of the column in the minimum volume of petrol. The column was eluted with a mixture of petroleum ether and diethyl ether, the amount of diethyl ether increasing from 1% to 20% in stages during the separation. The eluate was collected in 1000 ml batches and the solvent removed (RFE) with minimal heating to avoid oxidation. Each sample was checked by thin layer chromatography for the presence of oxidation products (slow running) and by gas chromatography for acid purity. Samples of sufficient purity (> 99.5% oleic acid) were combined and placed in a tightly sealed container under dry nitrogen. This was stored at approx. 5°C until required. If the storage time extended over several months, the oleic acid was analysed before use and passed down a further silica column if necessary.

In total 130 g oleic acid of > 99% purity by gas chromatography, and one spot on thin layer chromatography, was obtained. This material was a clear liquid at room temperature.

4.3 Preparation of Elaidic Acid

The isomerisation of the *cis* double bond of 18.1 (oleic) acid to produce a *trans* double bond can be catalysed by the use of nitrous acid. Nitrogenous by-products can be removed by silica column chromatography and recrystallisation and the percentage of *trans* acid formed can be

determined by using either IR spectroscopy or silver ion thin layer chromatography followed by gas chromatography with an internal standard.

4.3.1 Isomerisation

Oleic acid (36 g) was placed in a 3-necked flask fitted with a mechanical stirrer, an air condenser with drying tube and a dropping funnel. The flask was flushed with nitrogen gas and placed in an oil bath at 65°C. A solution of nitric acid (2 ml, 6 M) was added followed by sodium nitrite solution (2.4 ml, 2 M) and the mixture stirred vigorously. The temperature was maintained at 65°C for 30 mins after which the flask was cooled and the mixture extracted with diethyl ether. The ether extract was washed with water (2 x 50 ml) and the aqueous washings extracted with ether (3 x 50 ml) to recover the maximum amount of organic material. The combined ether solutions were dried (sodium sulphate) and the bulk of the solvent removed (RFE).

4.3.2 Purification

The solid reaction product was dissolved in the minimum of petroleum ether and passed down a silica (15 g, 2 cm i.d.) column. The column was eluted with petroleum and the clear eluate (175 ml) was collected. The solvent was removed (RFE) and the resulting white solid recrystallised three times from methanol (80 ml) at 0°C. The final methanol crystallisation yielded 15.5 g white crystals (m.p. 44-5°C, lit. 43-5°C[9]. The material remaining on the column was

flushed through with methanol and stored for possible incorporation into later batches of impure elaidic acid.

The analysis of the purified elaidic acid showed it to be > 99% *trans* isomer.

4.4 Glycerol 1-monostearate

4.4.1 Glycerol 1-monostearate was prepared via 1-stearoyl isopropylidene glycerol. The isopropylidene group afforded a useful blocking group to ensure acylation occurred at a terminal carbon atom of the glycerol (see equation below), and could be removed under conditions which would not encourage acyl migration.

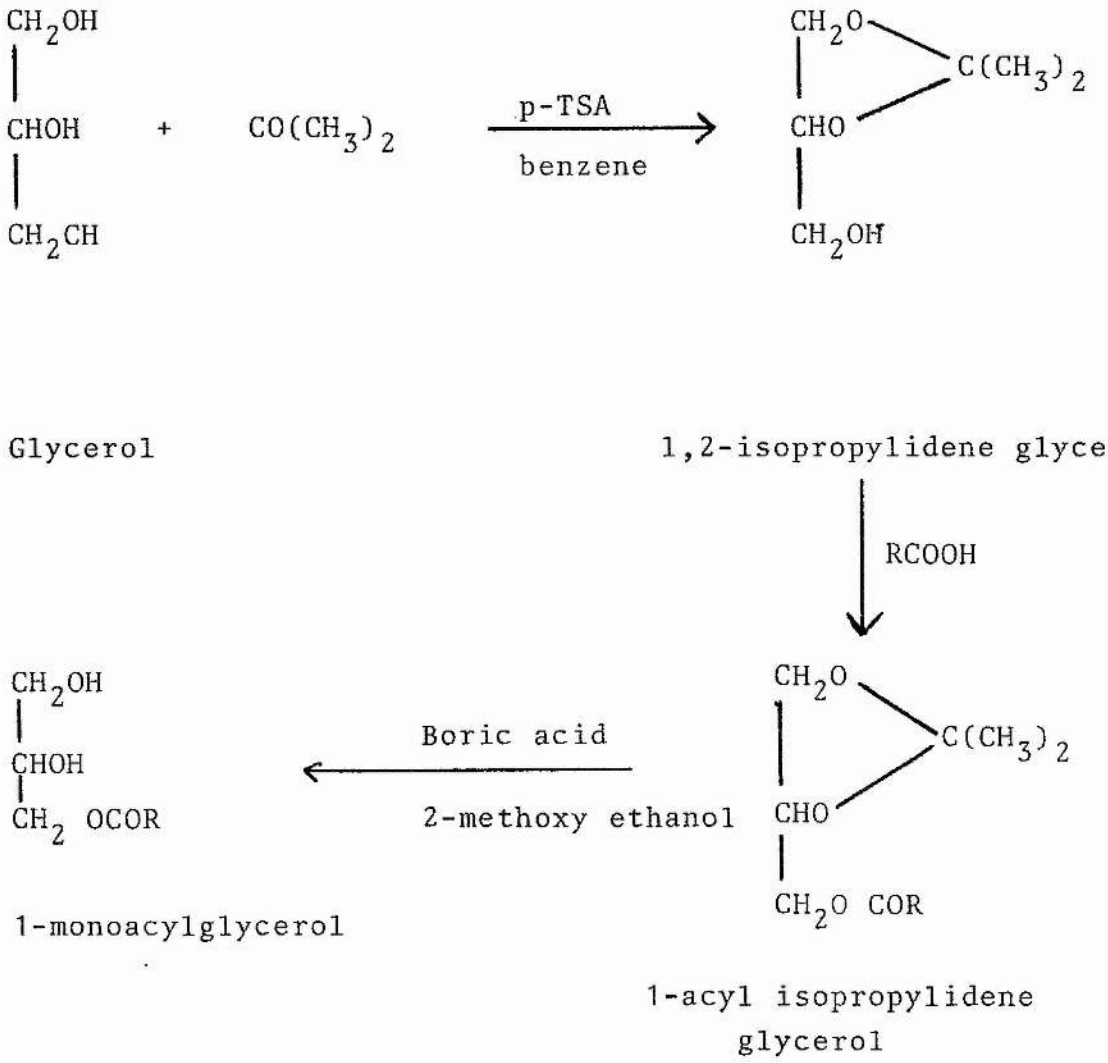


Figure 3

The monoacylglycerol was prepared in approx. 50 g quantities at any one time but this could not be conveniently scaled up due to the nature of the work-up.

4.4.2 1-stearoyl isopropylidene glycerol

Glycerol (66.5 g, 0.72 mole) was weighed directly into a 2 litre 3-neck round bottom flask and acetone (106 ml, 1.44 mole), benzene (500 ml) and p-toluenesulphonic acid (2 g) added. The flask was fitted with a Dean and Stark trap and the mixture refluxed for at least 10 hours (overnight). By this time all water-formation had ceased. The flask was allowed to cool and stearic acid (68.2 g, 0.24 mole) added. The mixture was again brought to reflux for at least 15 hours (overnight). When water formation had ceased sodium acetate (2 g) was added and the mixture left to cool. The mixture was transferred to a 2 litre separating funnel, washed with water (4 x 150 ml) and dried (sodium sulphate). The product solidified on cooling after removal of the solvent (RFE).

4.4.3 Glycerol 1-monostearate

The product obtained (above) was transferred to a 1 litre 3-neck round bottom flask. Boric acid (140 g) and 2-methoxyethanol (420 ml) were added and the flask fitted with a mechanical stirrer and air condenser. The mixture was heated on a steam bath for 1.5 hours after which it was cooled and transferred to a 5 litre separating funnel containing ethanol-free chloroform (freshly redistilled, 2.5 litres). It was then washed with water (3 x 1 litre),

dried (sodium sulphate) and the solvent removed at approx. 40°C (RFE). The solid residue was taken up in petrol (1 litre) and the mixture boiled. Not all the product dissolved. This was then allowed to crystallise at room temperature before being filtered and the solid washed with petrol (100 ml). The solid product was recrystallised from petrol/benzene (1:1 v/v, 1.5 litres) at 5°C over-night. The product (58 g, 67.5% yield) was analysed by thin layer chromatography (5% w/w boric acid with the silica) and by gas chromatography of the TMS ether. Analysis showed >99.5% purity, as required for the monoacylglycerol to be used for further reaction. M.p.t. = 81-82°C lit.[10].

4.5 Glycerol 1-stearate 3-palmitate

Glycerol 1-stearate (20 g, 0.056 ml) was stirred in a round-bottomed flask containing chloroform (240 ml, 12 x weight of monoacylglycerol) and pyridine (5.5 g, 0.07 mol). Palmitoyl chloride (19.2 g, 0.07 mol) was dissolved in chloroform (50 ml) and added dropwise over one hour from a dropping funnel fitted with a calcium chloride drying tube. The mixture was stirred for 24 hours at room temperature, after which the volatile materials were removed (RFE). The resulting solid was crystallised twice from petrol/ethanol (4:1, 360 ml) at 10°C overnight.

Analysis of the precipitate and the mother liquor by boric acid thin layer chromatography showed no presence of monoacylglycerol starting material. However, in addition to the desired diacylglycerol there appeared to be a

significant amount of triacylglycerol, presumably SPP, formed from complete acylation of monostearin by palmitoyl chloride. To effect the removal of the triacylglycerol, a further 5 crystallisations from petrol (400 ml) at room temperature were performed. Each precipitate and mother liquor were analysed by thin layer chromatography and showed a steady removal of the less polar triacylglycerol from the product. The final crystallisation showed no trace of triacylglycerol, and precipitate and mother liquor had identical, one spot, thin layer chromatography traces. The diacylglycerol (13.6 g, 0.02 mol) was dried over phosphorus pentoxide under vacuum and stored at 0°C until required (yield : 41%), mp 72-73°C (lit : 71°C)[11].

A further preparation of glycerol 1-stearate 3-palmitate followed the same process. Glycerol 1-monostearate (57.3 g, 0.16 mol) was reacted with palmitoyl chloride (54.8 g, 0.2 mol) in the presence of pyridine (15.8 g, 0.2 mol). The product (30 g, 0.05 mol, 31% yield) was analysed and stored as above (mp 71-72°C).

4.6 Glycerol 1-stearate 2-caprate 3-palmitate

Glycerol 1-stearate 3-palmitate (9.26 g, 0.016 mol) was placed in a 250 ml round-bottomed flask with pyridine (1.8 g, 0.023 mol) and chloroform (120 ml). The mixture was stirred and caproyl chloride (4.4 g, 0.023 mol) dissolved in chloroform (10 ml) was added dropwise over one hour. The dropping funnel containing the acid chloride was closed with a calcium chloride drying tube. The mixture was stirred at

room temperature for three days. Volatile materials were removed (RFE) and the solid product recrystallised from ethanol/acetone (9:1, 100 ml) at room temperature. A further two recrystallisations from acetone/petrol (4:1, 100 ml) at 0°C was followed by a final, petrol (150 ml) crystallisation before drying over phosphorous pentoxide under vacuum.

Analysis by thin layer chromatography of the final precipitate and mother liquor showed only one spot with no trace of starting material or free esters. The solid product was analysed by gas chromatography as shown.

Glyceride methanolysis	:	18.0 (40.9%); 16.0 (36.9%); 10.0 (22.1%) (by weight)
Theory	:	39.9%, 36.0%, 24.2%
Carbon no. gas chromatography	:	C-44 only
Lypolysis	:	10.0 (97.3%)
Total weight	:	9.1 g
Mp	:	56°C Literature mp [Me_2CO] : 55°C[12] .
Yield (from diacyl-glycerol)	:	77.4%

4.7 Glycerol 1-stearate 2-oleate 3-palmitate

Glycerol 1-stearate 3-palmitate (11.0 g, 0.019 mol) and pyridine (2.8 g, 0.036 mol) were stirred at room temperature. Oleoyl chloride (10.7 g, 0.036 mol) dissolved

in chloroform (50 ml) was added dropwise over one hour as described above (4.6). After three days with stirring the product obtained on removal of volatile materials was recrystallised twice from ethanol/acetone (9:1, 160 ml) at room temperature and twice from acetone/petrol (4:1, 160 ml) at 0°C. A final crystallisation from petrol (200 ml) at room temperature yielded 10 g precipitate which showed only one spot on thin layer chromatography. The mother liquor gave identical results. Analysis of the product gave:-

Methanolysis	:	16.0 (31.5%); 18.0 (34.7%); 18.1 (33.7%) (by weight)
Theory	:	16.0 (31.1%); 18.0 (34.5%); 18.1 (34.3%)
Carbon no. gas chromatography	:	C-52 only
Lipolysis	:	18.0 (0.6%); 18.1 (99.0%); 18.2 (0.2%)
Total weight	:	10.0 g
Mp	:	40°C Literature mp 37-8°C[11,13] (Me_2CO/hexane)
Yield (from diacyl-glycerol)	:	62.9%

4.8 Glycerol 1-stearate 2-elaidate 3-palmitate

Glycerol 1-stearate 3-palmitate (10 g, 0.017 mol) and pyridine (2.8 g, 0.036 mol) were stirred in chloroform (120 ml) and elaidoyl chloride (10.7 g, 0.036 mol) in chloroform was added dropwise as above (4.6). The reaction proceeded at room temperature for three days. On removal

of the volatile materials, the solid present was crystallised three times from ethanol/acetone (9:1, 140 ml) at room temperature, twice from acetone/petrol (4:1, 125 ml) at 0°C, and twice from petrol (100 ml) at room temperature. The final precipitate and the mother liquor showed identical, one spot, thin layer chromatography traces. Analysis gave:-

Methanolysis	:	16.0 (31.7%); 18.0 (35.2%); 18.1 (33.1%) (by weight)
Theory	:	16.0 (31.1%); 18.0 (34.5%); 18.1 (34.3%)
Carbon no. gas chromatography	:	C-52 only
Lipolysis	:	18.1 (98.3%)
Mp	:	54°C
Weight	:	10.9 g
Yield	:	75.7%

4.9 Glycerol 1-stearate 2,3-dimyristate

Glycerol 1-monostearate (28.6 g, 0.08 mol) was placed in a round-bottomed flask with pyridine (15.8 g, 0.2 mol) and chloroform (360 ml). The mixture was stirred and the myristoyl chloride (49.2 g, 0.2 mol) in chloroform (100 ml) added slowly as described (4.6). After three days at room temperature, the removal of volatile materials yielded a solid product which was recrystallised twice from ethanol/acetone (9:1, 500 ml) at room temperature, twice from acetone/petrol (4:1, 500 ml) at 0°C, and finally from petrol (300 ml). The precipitate (36.5 g, 0.05 mol)

and the mother liquor showed one spot on thin layer chromatography. The precipitate was dried and analysed. Analysis gave:-

Methanolysis	:	14.0 (59.7%); 18.0 (40.3%) (by weight)
Theory	:	14.0 (60.9%); 18.0 (39.1%)
Carbon no. gas chromatography	:	C-46 only
Lipolysis	:	14.0 (99.2%)
Mp	:	56°C, Lit mp 56°C [14]
Weight	:	36.5 g, EtOH/C_6H_6
Yield	:	58.6% (from monoacylglycerol)

4.10 Glycerol 1,3-dipalmitate 2-laurate

Dihydroxyacetone (11.3 g, 0.125 mol) was stirred in chloroform (470 ml) and pyridine (24 ml, 0.25 mol) under dry nitrogen in a one-litre three-necked round-bottomed flask. Palmitoyl chloride (68.5 g, 0.25 mol) was added dropwise over one hour and the mixture stirred for a further three hours at room temperature. Water (200 ml) was added and the chloroform layer separated. The aqueous layer was extracted with chloroform (2 x 100 ml), the combined chloroform extracts washed with water (1 x 200 ml) and dried over anhydrous sodium sulphate. On removal of the chloroform (R.F.E.) a solid product (66 g) was obtained and recrystallised three times from dichloromethane/ether (1:1). The precipitate from the final crystallisation (mp 82°C, lit mp 81-82°C, 8 g, 11%[15] was dried over phosphorous pentoxide and used directly for the reduction to diacylglycerol.

Sodium borohydride reduction of ketone

1,3-Dihydroxypropan-2-one 1,3-dipalmitate (8 g, 0.014 mol) was dissolved in tetrahydrofuran/benzene (5:1, 240 ml). Water (12 ml, 0.67 mol) was added with stirring and the temperature reduced to 5°C. Sodium borohydride (0.8 g,0.02mol) was added and the mixture stirred for 30 minutes. Water (100 ml) was added and the mixture extracted with chloroform (2 x 100 ml). The combined chloroform extracts were washed with water, dried over anhydrous sodium sulphate, and the solvent removed (R.F.E.). The white solid thus obtained was recrystallised from chloroform and dried over phosphorous pentoxide (7.7 g, 0.0136 mol, 97% yield from ketone), mp 71-73° C (lit mp 73-74°C from acetone/hexane)[11,16].

Acylation of glycerol 1,3-dipalmitate

Glycerol 1,3-dipalmitate (7.7 g, 0.0136 mol) was stirred in chloroform (95 ml) and pyridine (1.6 g, 0.02 mol). Lauroyl chloride (4.4 g, 0.02 mol) in chloroform (10 ml) was added dropwise as described (4.6). After stirring at room temperature for three days and removing the volatile materials, the solid product obtained was recrystallised twice from ethanol/acetone (9:1, 100 ml), twice from acetone/petrol (4:1, 100 ml) at 0°C, and finally from petrol (100 ml) at room temperature. The precipitate and the mother liquor were identical by thin layer chromatography. Analysis of the product gave:-

Methanolysis	:	12.0 (27.5%); 16.0 (72.5%)(by weight)
Theory	:	12.0 (28.4%); 16.0 (71.6%)
Carbon no. gas chromatography	:	C-44 only
Lipolysis	:	12.0 (99.1%); 16.0 (0.9%)
Mp	:	59°C; Lit mp 53.5-55°C (from $EtOH/C_6H_6$)[17]
Weight	:	6.6 g, 0.009 mol
Yield	:	64.7% (from dipalmitate).

CHAPTER 5

DISCUSSION

The procedures followed for the synthesis and analysis of the five triacylglycerols were, generally, effective in producing the required compounds and in determining their purity. Some initial problems were overcome only after considerable experience in performing some of the reactions and analyses. Some of these problems, and how they were finally solved, are discussed below along with a consideration of the procedures used.

General

For an effective synthetic route the number of steps to reach the final product should be kept to a minimum while the yield of each reaction should be maximised without detriment to the purity of the intermediates. Introduction of an impurity or biproduct can cause unsatisfactory results if not dealt with at the appropriate stage. This has special relevance to triacylglycerol synthesis where intermediates, biproducts and the desired product can often exhibit similar properties (see below). The necessity for purity determination and biproduct identification makes the ease and accuracy of analytical techniques an important factor in determining the success or failure of a synthetic route. The problems encountered in triacylglycerol synthesis can be grouped into four main categories.

i) Acyl migration

The possibility of migration of acyl groups between the 1-, 2-, and 3- positions of an acylglycerol must be considered throughout a synthetic route. If migration is not minimised where necessary, the positional purity of the products isolated cannot be assured. Not only should acyl migration be avoided during synthesis and purification but also in the analytical processes throughout the route. If the analysis of an acylglycerol does not afford protection from acyl migration then there is no guarantee that the results and deductions drawn can be relied on.

Migration can occur in triacylglycerols where high temperatures or catalysts are involved. Provided, however, that the conditions for reaction and purification steps yielding the triacylglycerol are not extreme, acyl migration of the final product should not be a problem. The greatest difficulty occurs with the isolation of positionally pure intermediates, such as mono- and di-acyl-glycerols, and their accurate analyses. The promotion of acyl migration during chromatography using silica has been reported [18], however this can be removed (or minimised) by the incorporation of boric acid into the adsorbent. All the thin layer chromatographic analyses performed on partial glycerides isolated during a synthesis therefore utilized a silica/boric acid (20:1) mixture for the plate coating.

The nature of the acyl chains to be incorporated in the triacylglycerols of this project afforded some protection from excessive acyl migration problems. The speed of migration is dependent on the type of acyl group. Short chain lengths, chain-branched groups and unsaturated chains all undergo more rapid migration in a mono- or di-acylglycerol molecule than long chains, straight chains or saturated chains respectively. Due to these properties, the preparation of glycerol 1-stearate 3-palmitate was via the glycerol 1-stearate rather than the glycerol 1-palmitate intermediate. Of the two triacylglycerols requiring unsaturated components neither required incorporation of the unsaturated chain until the last step of the synthesis by proceeding via the 1,3 diacylglycerol. Incorporation of the unsaturated acyl chain into a terminal, 1- or 3-, position would have provided more difficulty. The preparation of glycerol 1-stearate 2,3-dimyristate also afforded the advantage of the incorporation of the shorter, myristoyl, chains at the final stage. The fact that isolation of a diacylglycerol was unnecessary also benefited the ease of synthesis.

Glycerol 1,3-dipalmitate 2-laurate again involved the shorter chain length only at the final step of the synthesis and no mono-acylglycerol was involved in the route.

ii) Properties of products and intermediates

As has been mentioned earlier, the similar behaviour of acylglycerols as regards crystallisation, chromatographic and melting behaviour can cause problems during synthesis and analysis. This is especially true where positional isomers become mixed, either by reaction problems or acyl migration (see above). The principal method of avoiding problems of this kind is to attempt to find a synthetic route which will provide ease of separation of the desired product from any biproducts, and so to use or develop analytical methods to ensure the purity of reagents and products at each stage.

iii) Decomposition of unsaturated chains

The problem of accelerated acyl migration of unsaturated acyl groups has already been mentioned. An additional feature of incorporation of these types of chains is their susceptibility to oxidation under conditions which can occur in acylglycerol synthesis. The problem can be minimised by ensuring controlled reaction conditions and avoiding prolonged exposure to the atmosphere particularly at elevated temperatures. During addition of unsaturated acid chlorides to a reaction the heat of reaction was allowed largely to dissipate by dropwise addition with mixing. Oxalyl chloride was the chosen reagent for use with unsaturated acids for conversion to acid chlorides as it is reported to produce fewer biproducts than thionyl chloride [19]. Preparation of unsaturated acid

chlorides was never attempted using the reflux conditions used for saturated acids.

iv) Purity determinations

The problems associated with the analysis of synthetic intermediates and products have been indicated throughout this discussion when arising from specific cases. Some of the techniques used to overcome specific problems will be discussed here.

A major contribution to the analysis of acyl glycerols has been the recent advances in gas chromatography. During the analysis of samples reported earlier (Chapter 3) the techniques of g.c. have allowed accurate analysis of several series of different compounds. For example unsaturated fatty acids by separation of methyl esters, monoacylglycerols for positional purity using TMS ether derivatives, triacylglycerol carbon number analysis and positional purity of triacylglycerols when used in conjunction with an enzymic lipolysis and thin layer chromatography. It can, therefore, be seen immediately that gas chromatography is an essential element of many useful analytical procedures.

Thin layer chromatography was also used extensively as an analytical technique with the nature of the separation dictating the solvent system required and the type of adsorbent used on the plates. Incorporation of boric acid into silica to minimise acyl migration (and improve separation) and of silver nitrate for separation according

to unsaturation, afforded a necessary extension to the usefulness of this technique. Although certain gas chromatography columns now becoming available may provide remarkable separation of specific compounds, the cheapness and simplicity of a good t.l.c. system, while providing information of reasonable quality, still makes it a very viable technique for the compounds studied. The use of commercially prepared "HPTLC" plates can improve the accuracy of information when dealing with small percentage impurities.

P A R T I I

PHYSICAL PROPERTIES

CHAPTER 6

INTRODUCTION

An understanding of the behaviour of fats used in industry is obviously of interest in the areas of product improvement, process modification and the use of additives. In addition, certain fat systems of commercial importance provide problems for which a direct solution is required. The phenomena of "bloom" in chocolate[20-23] and the grainy texture which can occur in margarine[24,25] are two examples. Any attempt to more fully understood the nature of these effects and how fats may be handled and modified without loss of desired characteristics requires an initial investigation of triacylglycerol crystallisation behaviour and solid state interactions. A comprehensive knowledge of how particular triacylglycerols will behave both in isolation and in mixtures with other triacylglycerols may allow prediction of natural fat system behaviour. This, in turn, may allow the modification of cheaper fats in order to obtain the characteristics, and therefore some of the uses, of more expensive materials. This has already been done to some extent with the hydrogenation of unsaturated fats and also by fractionation.

For the purposes of this study, the triacylglycerols were chosen to provide material with positional and chemical variation which may be of use for investigation of chocolate-like substances. The behaviour of triacylglycerols with *cis* and *trans* double bonds is an extension of this. The *trans* isomers are not generally found in natural fat systems, however,

catalytic hydrogenation of *cis* double bonds can result in their formation. The incorporation of triacylglycerols containing *trans* unsaturation into a fat system may cause significant changes to that system's characteristics. This factor would require to be considered carefully in determining the suitability of a component for incorporation into a fat mixture.

The triacylglycerols predominant in cocoa butter contain an oleic acid moiety in the 2-position. The nature of the 2-position interactions in mixed systems is therefore of great interest. For this reason, in association with the *cis/trans* interactions mentioned above, POS and PES were two of the triacylglycerols chosen for study. The characteristics of a triacylglycerol with a short chain in the 2-position were also of interest and so PCS was prepared for examination. PLP provided an interesting symmetrical triacylglycerol with a further chain difference in the central position while SMM would be expected to provide a very different structural pattern having a very different chain length distribution to the other triacylglycerols.

The properties of cocoa butter itself, and its behaviour in chocolate systems, have been studied using a variety of methods [26-32,21] however, the principles used as a basis for triacylglycerol characterisation in this study are discussed below.

Triacylglycerol molecules in the solid state pack in several different ways to provide distinct crystal structures. These different forms are called polymorphs and exhibit

individual thermal properties in addition to their distinct X-ray diffraction patterns. The properties of different polymorphs can, therefore, be used as a method of characterisation using combined techniques such as differential scanning calorimetry (DSC) and X-ray diffraction data. The use of DSC provides accurate information concerning the thermal properties of a polymorph (eg. melting point, solidification point, heats of fusion and crystallisation etc.) while X-ray powder diffraction data confirm a particular crystal packing type. The criteria for assigning a particular crystalline form to a polymorph are given below.

6.1 Simple Packing Arrangements in Crystalline Triacylglycerols

Two criteria are generally used to denote a crystal type for triacylglycerol polymorphs. These are termed the long and short spacing and are taken directly from the X-ray powder diffraction pattern. A single strong spacing at ca. 4.15 Å characterises the α structure. Two strong spacings ca. 3.8 Å and ca. 4.2 Å or three strong spacings ca. 4.27 - 3.97 and 3.71 Å, and also shows a doublet around 720 cm^{-1} in the infrared spectrum, characterises a β' polymorph. If the diffraction pattern fits neither of the above criteria then the structure is termed β [33].

These characteristic short spacings are independent of the chemical composition of the triacylglycerol and occur with other long chain compounds. This suggests that the spacings are related to the packing of long hydrocarbon chains, in particular the cross sectional packing (Fig. 4).

The second piece of information used to denote crystal type is the long spacing. This arises from the repeating layers of triacylglycerol molecules. Thus the long spacing of a triacylglycerol depends on its effective length (presence of unsaturated chains can cause this to change radically depending on the stereochemistry) and on the angle at which the chains are tilted. Each of the β' and β modifications have varying angles of tilt, however, the α form has all the chains packed at right angles (90°) to the end group plane.

The thickness of the layers can be related to the presence of either two or three acyl chains thus giving the terms double and triple chain length packing (DCL and TCL respectively). This additional structural information can be added to the previous symbols as a suffix. Thus a polymorph showing a double chain length layer thickness (long spacing usually ~30-40 Å depending on the effective chain lengths) would be termed β-2 (Fig. 5).

In general the α form is obtained by fast cooling of the melt and has the lowest melting point and density. The β form has the highest melting point and density and the β' form has intermediate values. Transformations occur in the order $\alpha \rightarrow \beta' \rightarrow \beta$ and are not reversible. A simple representation of possible transitions between the polymorphs of a triacylglycerol is given in Fig. 6.

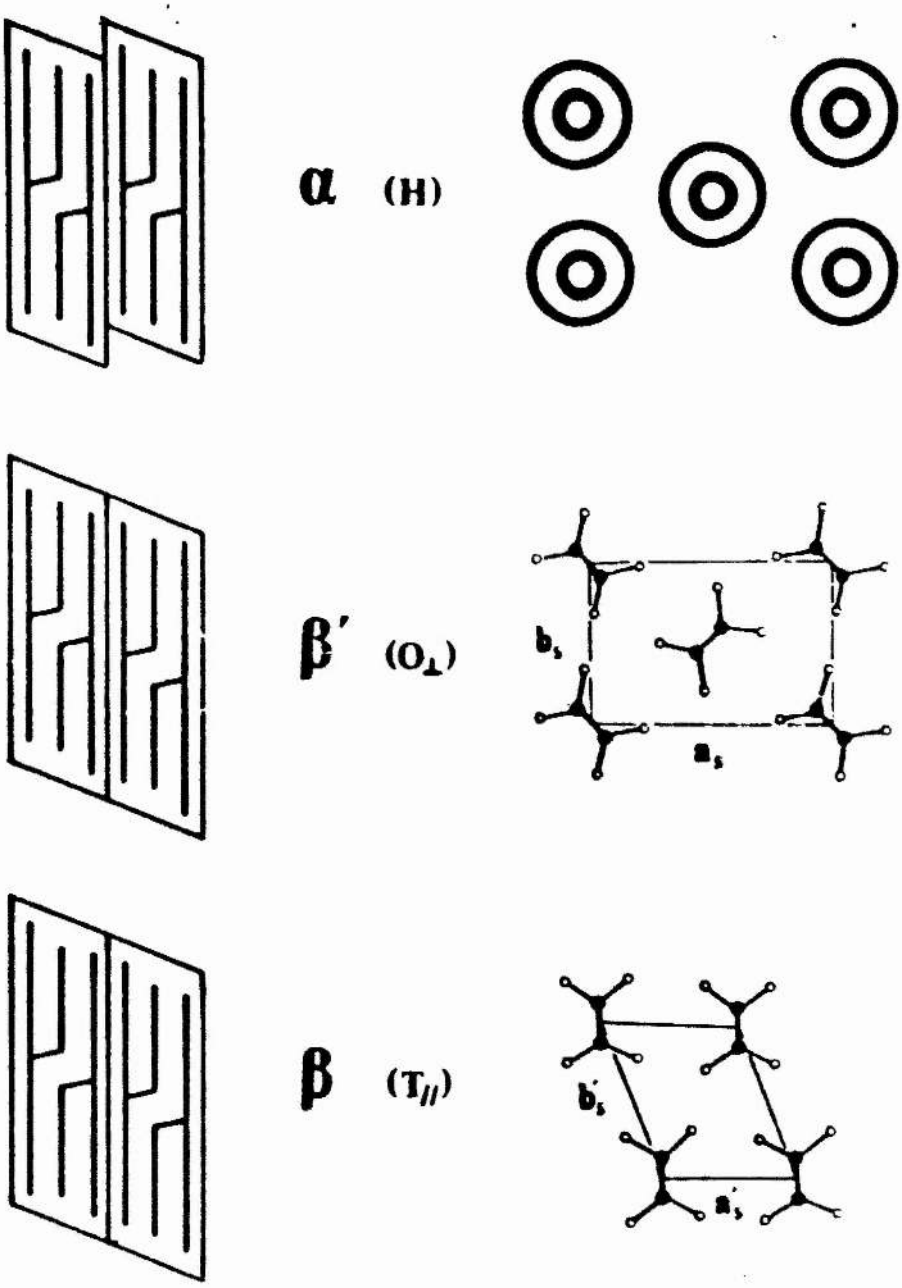


Fig. 4

Schematic orientation of the proposed triglyceride dimers of the α-, β′- and β-forms showed together with respective chain packing subcell. (34)

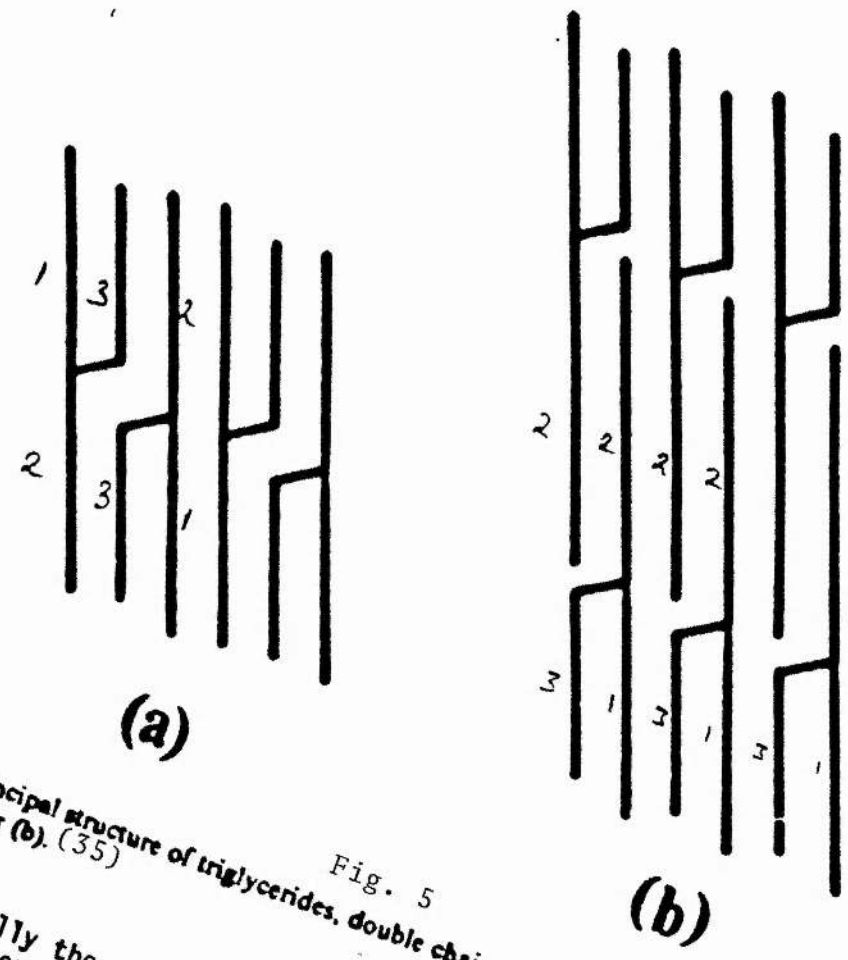


Fig. 5

Principal structure of triglycerides, double chain layer (a) and triple chain layer (b). (35)

Generally the central acyl chain (chain no. 2) points away from the two external chains (1 and 3).

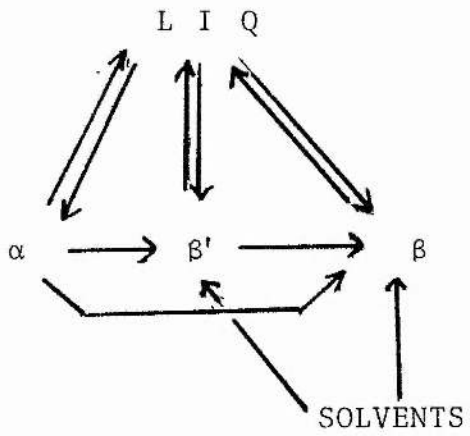


Fig. 6

Possible polymorphic transitions
of a triacylglycerol.

6.2 The Use of Differential Scanning Calorimetry

The different crystalline forms discussed above exhibit different melting points and related thermal properties. Differential scanning calorimetry is a technique which records the energy required to establish zero temperature difference between a sample and a reference (usually an empty sample holder), while the system is subjected to controlled heating or cooling. Thus as a sample melts or crystallizes, the heat of fusion or crystallisation causes its temperature to change relative to the reference. This is recorded as heat flow necessary to re-establish zero temperature difference and can be plotted versus the temperature of the system[36,37].

This allows the study of the melting and crystallisation behaviour of a sample under controlled conditions. It is limited, however, to thermal measurements and gives no direct information on the nature of the polymorphic types present. It must, therefore, be combined with X-ray powder diffraction data where possible .

When examining DSC thermograms it should be noted that polymorphic transitions occurring during heating can proceed in three ways: i) complete melting of the lower form followed by crystallisation to a higher form, ii) partial melting of the lower form before conversion to a higher form, or iii) with no melting at all [38-41].

When complete melting occurs followed by crystallisation, the endothermic peak (below base-line) gives a measure of the heat of fusion (ΔHfus) of the polymorph which has melted. This is followed by an exothermic peak (above base-line) which is a measure of the heat of transition and crystallisation to the higher melting form. Partial melting accompanied by formation of a higher polymorph can result in both endothermic and exothermic changes being incorporated into the same peak. When this occurs the peak area would be a measure of the difference between heat absorbed in melting and the heat emitted during formation of the higher polymorph. If no melting at all takes place during a transition then only an exothermic change is likely in which case the exothermic peak area is a measure of the heat of transition. This choice of possibilities complicates attempts to use DSC alone to obtain

quantitative thermodynamic data such as heats of fusion unless the nature of the transition studied is well understood.

During DSC analyses of triacylglycerols, the 'thermal history' of the sample is important due to the possibility of preferential formation of different polymorphic forms when subjected to certain temperature conditions. The crystal structure present at any particular time depends on the rate of heating and cooling, the temperature at which the sample is maintained and the length of time held at that temperature. However, for the purposes of this project, it was assumed that once a triacylglycerol was taken beyond its melting point for at least 10 minutes, all structural patterns were broken down. Consideration of thermal history therefore begins from when the sample is subjected to temperatures below its melting point. Samples of a particular triacylglycerol were considered to be identical and without a crystalline nature when in the molten form. The possibility of a persistence of "structural memory" after melting[34] was not investigated. Similarly, the existence of "sub-α" forms, which may undergo reversible transformation to an α form, were not considered.

Previous studies of binary systems of fats and their phase behaviour have employed a wide variety of techniques[42-52] This makes comparisons between the data difficult. Melting behaviour has often been presented without supporting polymorphic classification by X-ray diffraction. This severely limits the uses of the data as the polymorphic forms involved

may be open to question. Other methods[53] have made use of elaborate conditioning procedures to prepare stabilised samples. For this study the method employed was hoped to provide a fairly rapid technique to supply sufficient data to compare the compatibilities of pure triacylglycerols in known polymorphic forms. The experiments were not designed to provide a full survey of the complexities of the polymorphic interactions or detailed phase behaviour.

CHAPTER 7

EXPERIMENTAL/RESULTS

7.1 General Methods and Instrumentation

The physical properties of triacylglycerols are generally discussed in terms of the experimental data available from Differential Scanning Calorimetry (DSC), X-ray crystallography and wide-line or pulsed nuclear magnetic resonance. The facilities of the Cadbury Schweppes Lord Zuckerman Research Centre were made available for the study of some aspects of the physical properties of the triacylglycerols synthesised in St. Andrews.

Studies were made on the five triacylglycerols prepared as set out in Part 1. In addition, the laboratory had in stock a selection of 'Sigma' saturated mono-acid triacylglycerols and four triacylglycerols prepared in St. Andrews by Dr. C.M. Scrimgeour (PEE, PSO, MLS and LMS). The mono-acid triacylglycerols were useful materials for familiarisation with the techniques due to the availability of published data (e.g. Appendix 4).

All the synthesised triacylglycerols were studied to determine their individual melting and crystallisation characteristics by DSC. A study of the phase behaviour of certain binary mixtures was carried out using some of the five triacylglycerols prepared in Part I, and in one case the previously prepared PEE was also used.

The results of these studies, giving brief experimental conditions, are given in sections 7.4 and 7.5. A more detailed explanation of the experimental data, and the reasons behind the procedures used, is given in the Discussion. Given below is a summary of the equipment used.

Instrumentation

Differential scanning calorimetry

The laboratory was equipped with a Du Pont 1090 Thermal Analyzer providing disc memory and microcomputer analysis. The data output for the 1090 was generally obtained in the form of a melting or crystallisation thermogram, i.e. a plot of heat flow versus temperature. It was then possible to select individual peaks on the graph for further computation as required, e.g. heats of fusion or crystallisation (the extrapolated area between curve and base-line) and peak minimum or maximum. The peaks on the thermogram indicate whether the change occurring is either exothermic giving a peak above the base-line (e.g. crystallisation) or endothermic giving a peak below the base-line (e.g. melting). This notation is sometimes reversed in the literature where different instrumentation has been used.

X-ray powder diffraction

Two cameras were used for crystallography in order to accommodate the range of angle of scattering which was necessary for structure determination.

The short spacing of a sample was recorded on a strip of film and once developed the diffraction pattern was projected to paper by a recording microdensitometer. The long spacing was recorded by a Rigaku Denki camera producing a chart printout of signal intensity from a Geiger counter versus time (angle of diffraction)(see App. 6 for specifications).

7.2 Single Triacylglycerols

All the triacylglycerols were subjected to an initial temperature programme to determine melting point and the heat of fusion. The programme consisted of a 5°C/min rise from 20°C to above the melting point (70° or 90°C). These triacylglycerols were examined as obtained from the synthetic route, or as supplied from Sigma, and were termed 'solvent-crystallised' materials as distinct from materials crystallised from the melt or by thermal conditioning. In some cases the polarity of the solvent used for the crystallisation was a factor in the crystalline form obtained and the solvent system used is, therefore, sometimes detailed.

Melting behaviour of each triacylglycerol was then studied by subjecting samples to a range of temperature conditions dependent on the individual melting points and the details of the DSC scans as they were obtained. A sample of cocoa butter was also tested in a similar manner. In these studies heating and cooling rates were 5°C/min. The scan conditions, thermal history and details of the DSC plot are reported later (pages 64 - 82).

Some additional scans were obtained using other heating and cooling rates. The results of these scans are reported with reference to the melting points and heats of fusion and compared with literature data when available.

Samples of MLS, LMS, PEE and PSO were also left to crystallise from melt by standing at room temperature. This provided an immediate comparison between the stable, solvent-crystallised polymorph and the form preferred on 'natural' cooling rather than from a temperature controlled DSC scan. These samples were studied by X-ray powder diffraction (see later), however MLS and LMS samples were also studied by DSC scan.

7.3 Binary Systems

Binary mixtures of triacylglycerols were prepared to cover a 0-100% composition range in approximately 10% steps. This provided an overview of the phase behaviour of the system after which any areas of composition showing interesting or unusual effects could be studied in more detail, using samples with smaller composition changes.

Samples were prepared in approximately 1 g amounts (total weight) in glass vials. The vials were placed in an oven pre-heated to >90°C for 15-20 minutes. After removal from the oven the vials containing samples were allowed to cool and the samples crystallised under varying controlled conditions. This control was effected by

placing the vials into a metal block suspended in a water bath. The temperature quoted for the conditioning of samples refers to the temperature of the water surrounding the metal block. The water baths were always held at the desired temperature for at least one hour before use to ensure a constant temperature. The glass vial containing the sample was a snug fit with the sides of the metal block to avoid air circulation and temperature variation around the sample. The water level against the block was kept as high as possible to maximise the temperature stability of the block and sample.

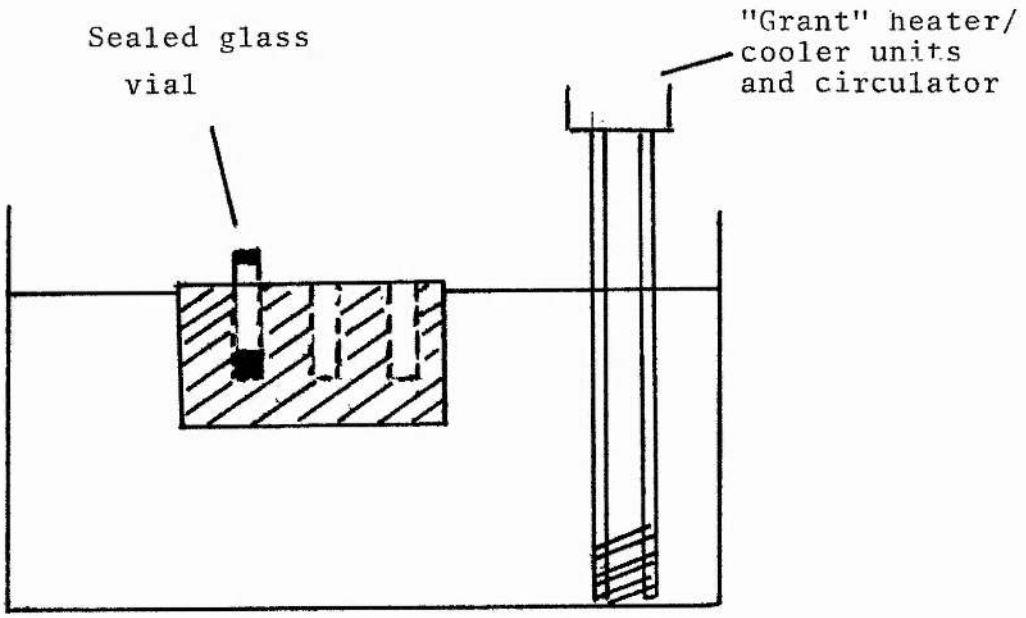


Fig. 7

The thermal conditioning of each series of samples, and the resulting observations, are given in detail as a change in conditions could alter the preferred polymorphic

form and therefore the phase behaviour and compatibility of the triacylglycerols would change. Compatibility comparisons would then be difficult without the full conditioning sequence being known.

The composition of all binary mixtures in this work are given in mole percentages unless otherwise stated.

Initial conditioning

To obtain data on easily-formed polymorphic forms and to get some idea of stability, melts were cooled directly to 15°C and stabilised at this temperature for 15 hours before testing. The sample was powdered and a small amount weighed into a DSC pan and subjected to a 15°C-90°C scan at 5°C/min. A larger amount was taken for X-ray powder diffraction analysis - both long and short spacings being measured. The X-ray experiments were always performed at room temperature (~23°C). Melting points and the polymorphic forms indicated by X-ray diffraction data were plotted on a composition vs. temperature plot for comparison with later experiments.

Further conditioning

The information obtained from the above procedure carried out for all the binary mixtures to be studied, was combined with data from the study of the individual triacylglycerols. This allowed a specific sequence of temperature conditioning to be devised for each system which would attempt to obtain the most stable polymorphic formation over an entire composition range. The nature of

the required conditioning, its effectiveness in producing a given polymorph and the melting behaviour and change in X-ray pattern with composition were all useful criteria for discussing the nature of the compatibility of two triacylglycerols.

On obtaining the "stabilised" series of samples for a given binary system, the samples were then maintained at a temperature 2-3°C below the minimum melting point and their melting point analysed periodically to ensure no further transitions were occurring over prolonged (several weeks) storage.

Examples of some DSC scans and X-ray long and short spacings are given in Appendix 3. These have been chosen to illustrate α,β' and β short spacing patterns and the differences in long spacings between double packing, triple packing and an α structure where chains are not tilted.

The DSC scans show the three melting points for the α, β' and β forms of PES. The different melting points for solvent and melt crystallised β-3 PCS are also included. X-ray patterns for these samples, by both cameras used, are also given as examples.

7.4 RESULTS

FOR SINGLE TRIACYLGLYCEROLS

X-ray Crystallography of Single Triacylglycerols

Triacylglycerol		Purity (%)	Method of crystallisation*	Long spacing (Å)	Crystalli form
1.	POS	98+	Solvent	63.1	β-3
	POS	98+	Melt	65.9,33.6	ambiguous
2.	PES	98+	Solvent	44.2	β-2
	PES	98+	Melt	47.8	
3.	PCS	98+	Solvent	58.9	β-3
	PCS	98+	Melt	60.9	β-3
4.	PLP	98+	Solvent(non polar)	38.2	β-2
	PLP	98+	Solvent (polar)	61.7	β-3
			Melt	43.1	
5.	SMM	98+	Solvent(non polar)	41.1	β-2
	SMM	98+	Solvent (polar)	64.0	β-3
	SMM	98+	Melt	44.6	
6.	MLS	98+	Solvent	60.5	β-3
	MLS	98+	Melt	60.1	β-3
7.	LMS	98+	Solvent	38.2	β'-2
	LMS	98+	Melt	39.4	β'-2
8.	PEE	98+	Solvent	44.2	β-2
	PEE	98+	Melt	44.4	β'-2
9.	PSO	98+	Solvent	68.5	β'-3
	PSO	98+	Melt	39.8	α
10.	SSS	90+	Solvent	44.8	β-2
	SSS	90+	Melt	51.7	α
11.	PPP	90+	Solvent	40.7	β-2
	PPP	90+	Melt	46.0	α
12.	AAA	99	Solvent	49.4	β-2

* Samples from 'melt' were obtained by placing solvent-crystallised material in an oven at 100°C until molten and the standing at room temperature for 1-4 hours.

Non polar solvent crystallisation involved dissolving the material in 40/60 petroleum ether and standing at 5°C overnigh

Polar solvent crystallisation involved dissolving the sample in the minimum of chloroform followed by the addition of methanol until the solution became cloudy.

Crystallisation was allowed to occur overnight at room temperature.

Endothermic Transitions Observed by DSC

Triacylglycerol: POS Purity >98% Sample size: 6.4 mg
Scan rate: 5° C/min

Run No.	Temp. range of scan (°C)	Time Isothermal at lower temp. (mins)	Melting points (°C)
1	25 → 70		39.6
2	12 → 70	10	26.6
3	12 → 70	30	26.6
4	20 → 70	10	no peaks observed
5	20 → 70	30	32.9

Triacylglycerol: PES Purity >98% Sample size: 7.4mg
Scan rate: 5° C/min

Run No.	Temp. range of scan (°C)	Time Isothermal at lower temp. (mins)	Melting points (°C)
1	25 → 70		53.9
2	24 → 70	10	37.0,48.1
3	24 → 70	30	37.1,48.1

Triacylglycerol: PCS Purity >98% Sample size: 7.1mg
Scan rate: 5°C/min

Run No.	Temp. range of scan (°C)	Time isothermal at lower temp. (mins)	Melting points (°C)
1	25 → 80		56.6
2	20 → 80	10	53.5
3	20 → 80	30	53.5

Triacylglycerol: PLP Purity >98% Sample size: 5.5mg

Scan rate: 5° C/min

Run No.	Temp. range of scan (°C)		Time isothermal at lower temp. (mins)	Melting points (°C)
1	20	80	polar solvent	55.8
2	20	80	Non-polar solvent	52.6
3	24	80	10	32.4, 49.4
4	24	80	30	32.5, 49.4

Triacylglycerol: SMM Purity >98% Sample size: 10.2 mg

Scan rate: 5° C/min

Run No.	Temp.range of scan (°C)		Time isothermal at lower temp. (mins)	Melting points (°C)
1	25	80	polar solvent	59.3
2	25	80	Non-polar solvent	56.6
3	19	80	10	40.9,51.4
4	19	80	30	41.0,51.4

Triacylglycerol: MLS Purity >98% Sample size 4.8 mg
Scan Rate 5°C/min

Run No.	Temp. Range of Scan (°C)	Time Isothermal at lower temp. (mins)	Melting points (°C)
1	20 → 70		57.3
2	20 → 70	10	29.4, 54.2
3	30 → 70	30	54.2
4	32 → 70	30	54.0
5	35 → 70	30	no peaks observed
6	20 → 70	X-ray sample	54.9

Triacylglycerol:LMS Purity >98% Sample size 8.3 mg
Scan Rate 5°C/min

Run No.	Temp. Range of Scan (°C)	Time Isothermal at lower temp. (mins)	Melting points (°C)
1	20 → 70		52.6
2	20 → 70	10	29.0, 42.6
3	30 → 70	10	42.9
4	-20 → 70	10	29.4, 42.6, 47.4
5	-10 → 70	10	29.3, 42.6, 47.4
6	0 → 70	10	29.3, 42.5, 47.4
7	0 → 70	30	28.9, 42.0, 47.1
8	0 → 70	120	29.4, 42.3, 47.9
9	20 → 70	X-ray sample	45.1

cont./ ...

Triacylglycerol: PEE Purity >98% Sample size 10.1 mg
Scan Rate 5°C/min

Run No.	Temp. Range of Scan (°C)	Time Isothermal at lower temp. (mins)	Melting points (°C)
1	10 → 70		39.2
2	10 → 70	2	23.3, 34.4
3	20 → 70	10	23.9, 34.8
4	30 → 70	10	no peaks observed
5	30 → 70	30	no peaks observed
6	25 → 70	10	no peaks observed
7	25 → 70	15	31.3
8	25 → 70	20	32.6
9	25 → 70	30	32.9

Triacylglycerol: PSO Purity >98% Sample size 9.8 mg
Scan Rate 5°C/min

Run No.	Temp. Range of Scan (°C)	Time Isothermal at lower temp. (mins)	Melting points (°C)
1	20 → 70		42.7
2	20 → 70	10	27.6
3	30 → 70	10	no peaks observed
4	30 → 70	20	no peaks observed
5	25 → 70	10	no peaks observed
6	35 → 70	30	no peaks observed
7	25 → 70	20	26.8
8	10 → 70	10	20.8, 27.1
9	20 → 70	Overnight at RT	26.8, 31.7, 36.7
10	10 → 70	30	21.9, 27.5
11	5 → 70	10	21.4, 27.4
12	0 → 70	10	20.9, 27.4
13	-5 → 70	10	20.4, 27.6
14	-20 → 70	10	20.4, 27.8
15	20 → 70	Weekend	26.5,30.5,31.9,37.2
16	5 → 70	60	22.4, 29.1, 35.8
17	-20 → 70	30	20.8, 29.2, 36.1

Triacylglycerol: SSS Purity 90%+ Sample size 5.3 mg
Scan Rate 5°C/min

Run No.	Temp. Range of Scan (°C)	Time Isothermal at lower temp. (mins)	Melting points (°C)
1	20 → 90		69.3
2	20 → 90	10	54.4
3	0 → 90	10	54.6
4	30 → 90	10	54.7
5	30 → 90	45	54.8
6	40 → 90	10	55.1
7	50 → 90	10	55.2
8	20 → 90	RT for 3 days	54.9

Triacylglycerol: AAA Purity 99% Sample size 4.9 mg
Scan Rate 5°C/min

Run No.	Temp. Range at Scan (°C)	Time Isothermal at lower temp. (mins)	Melting points (°C)
1	20 → 90		79.7
2	20 → 90	10	63.7, 75.9
3	0 → 90	10	63.8, 76.0
4	50 → 90	10	63.6, 75.1
5	20 → 90	Overnight at RT	62.9, 74.8
6	-10 → 90	10	63.4, 75.5
7	-30 → 90	10	63.6, 75.7
8	-50 → 90	10	63.7, 75.7

cont./ ...

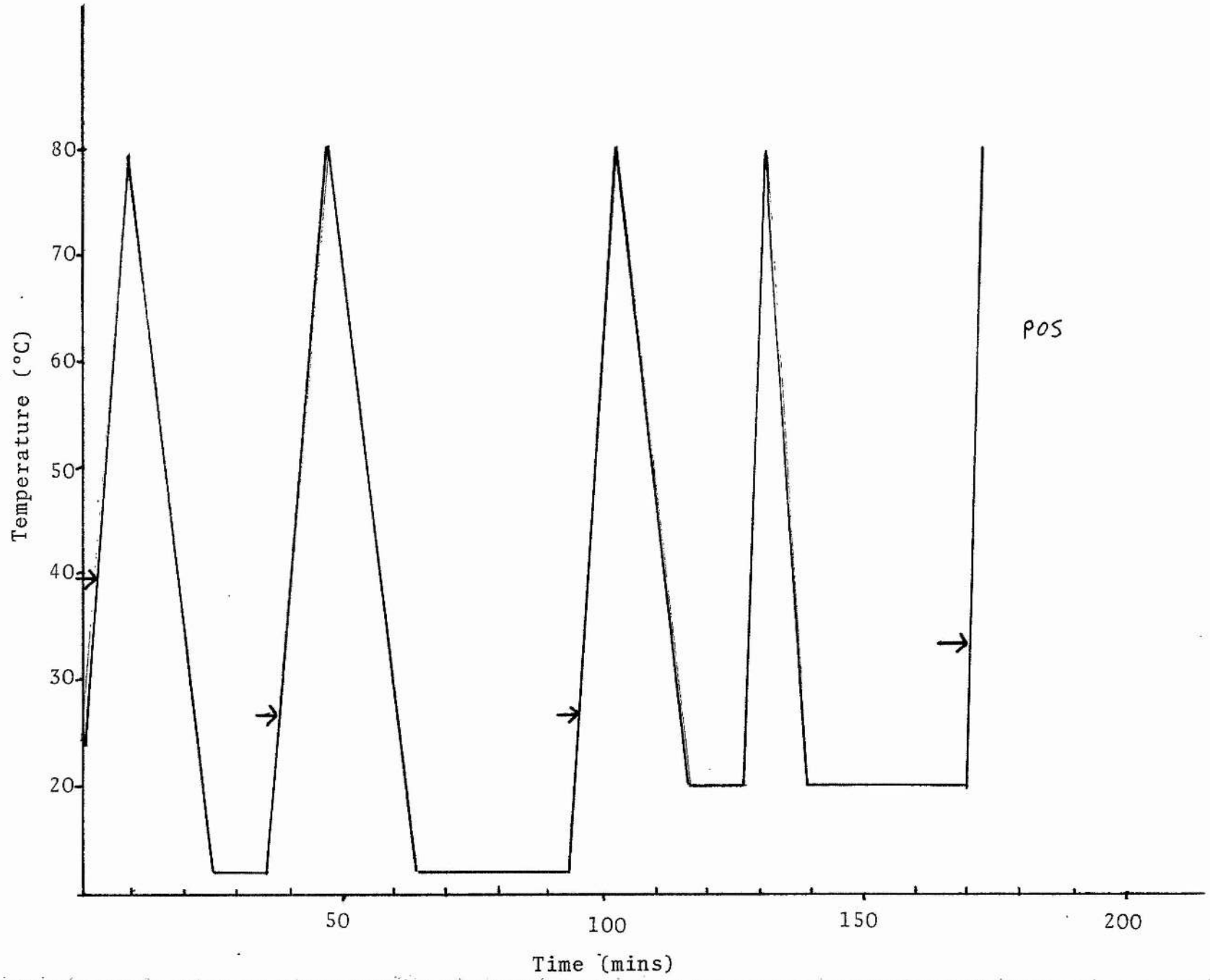
Cocoa Butter Sample size 10.6 mg Scan Rate 5°C/min

Run No.	Temp. Range of Scan (°C)	Time Isothermal at lower temp. (mins)	Melting points (°C)
1	15 → 70		33.9
2	15 → 70	10	22.0
3	20 → 70	10	24.7 (small peak)
4	25 → 70	30	none
5	-10 → 70	10	21.2

THERMAL HISTORIES OF TRIACYLGLYCEROLS
IN A SERIES OF DSC SCANS

$\longrightarrow$ indicates endothermic phase transition

$\Longrightarrow$ indicates exothermic phase transition.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30
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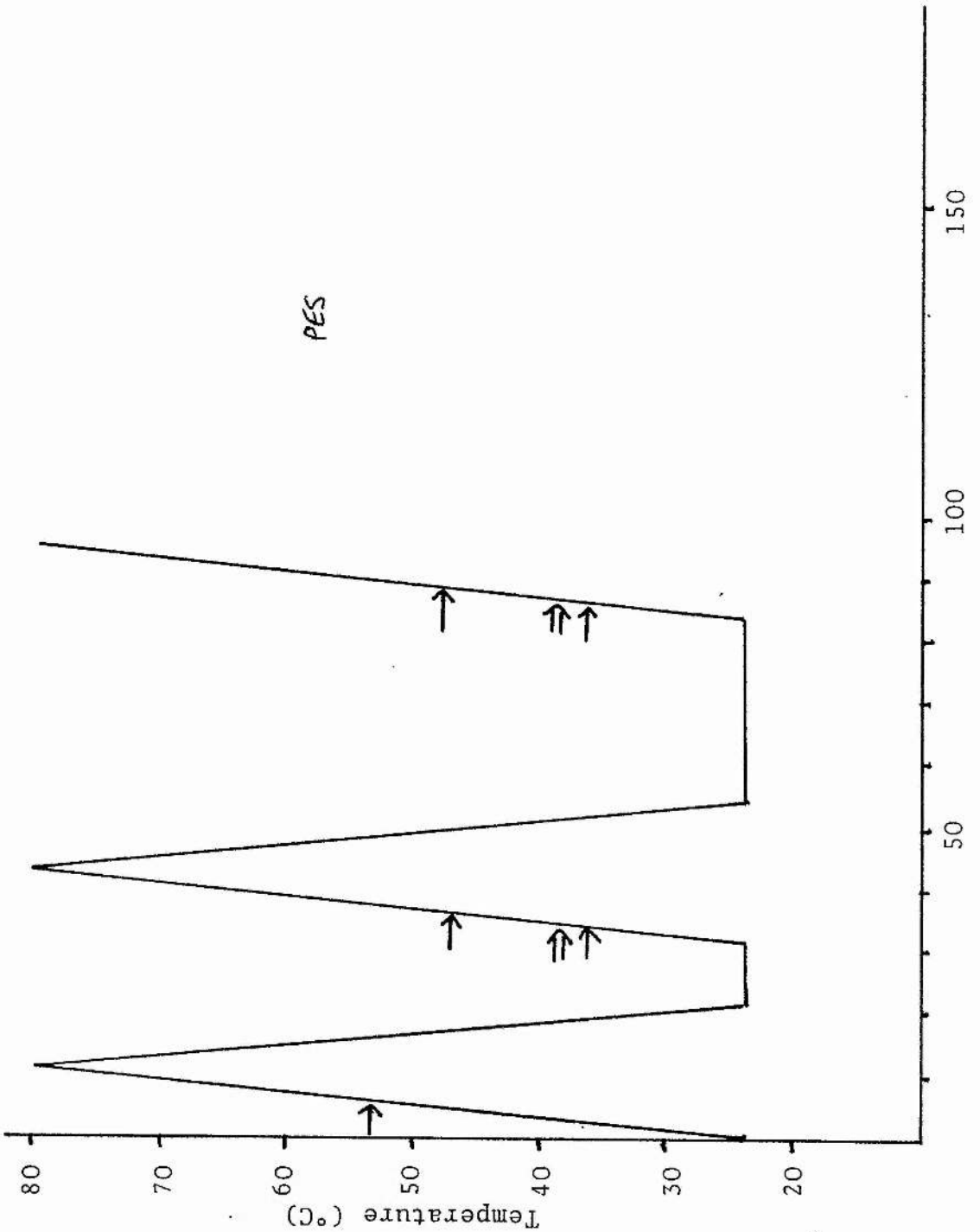
200
Time (mins)
POS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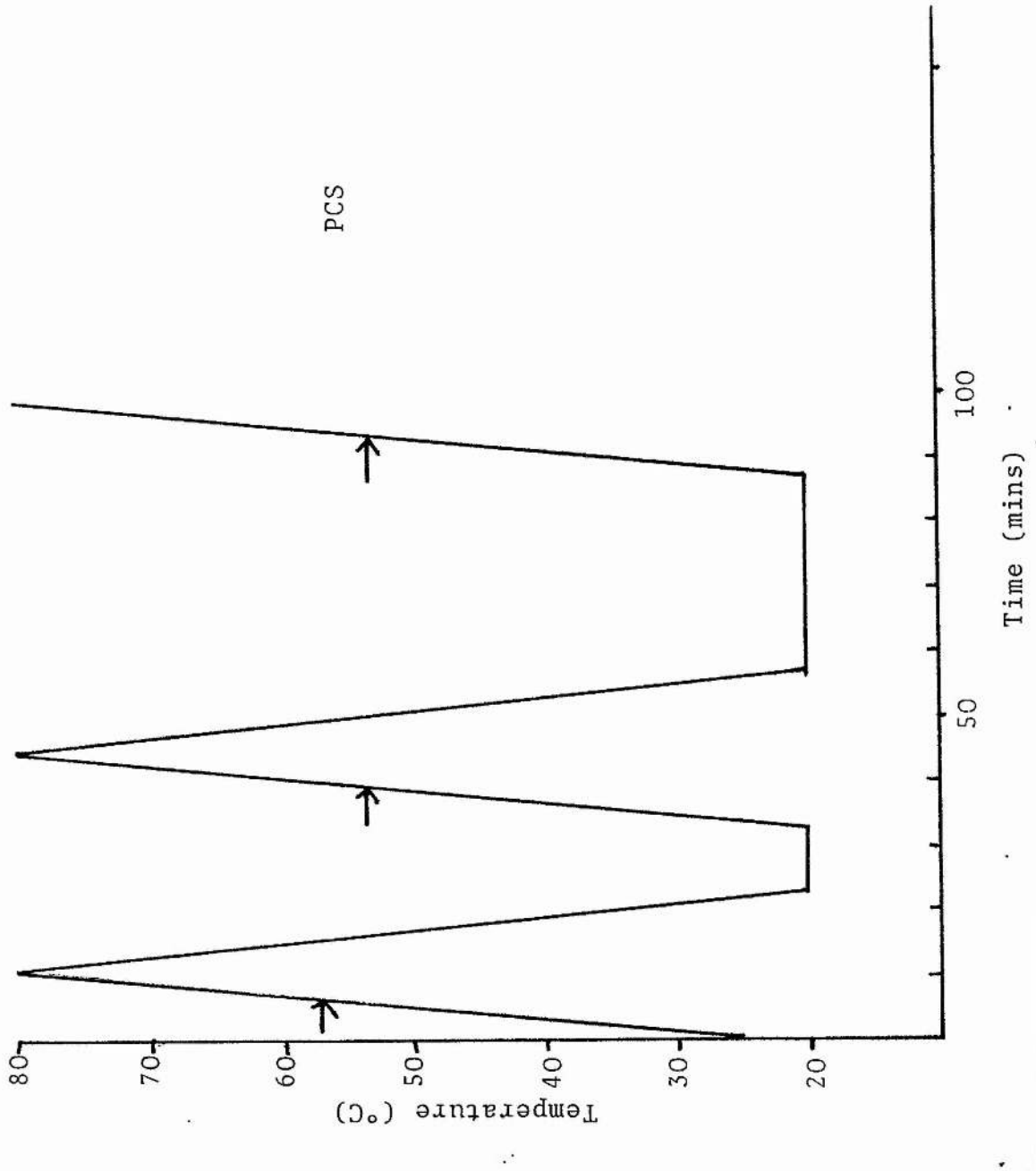
Time (mins).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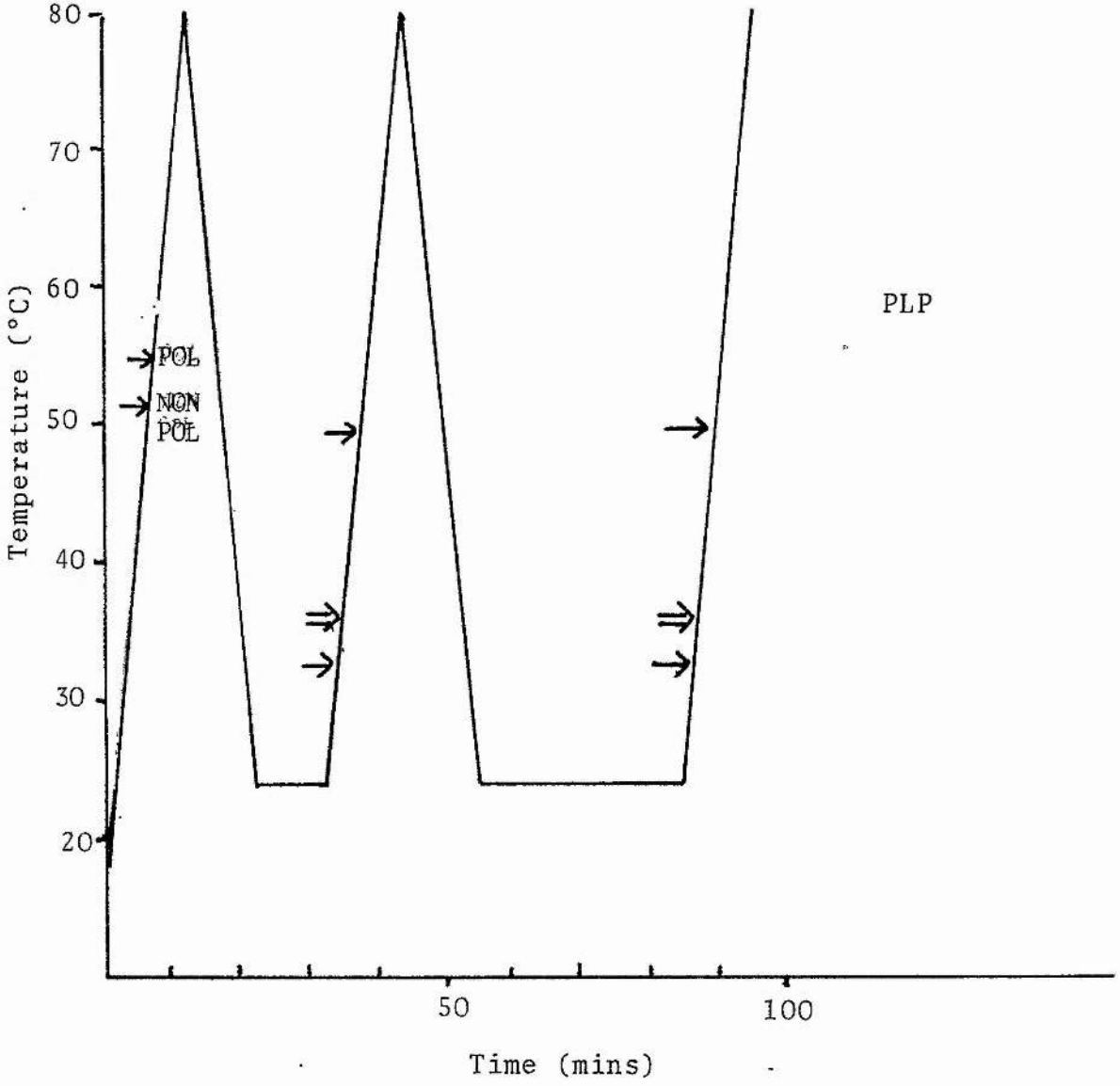
Time (mins)



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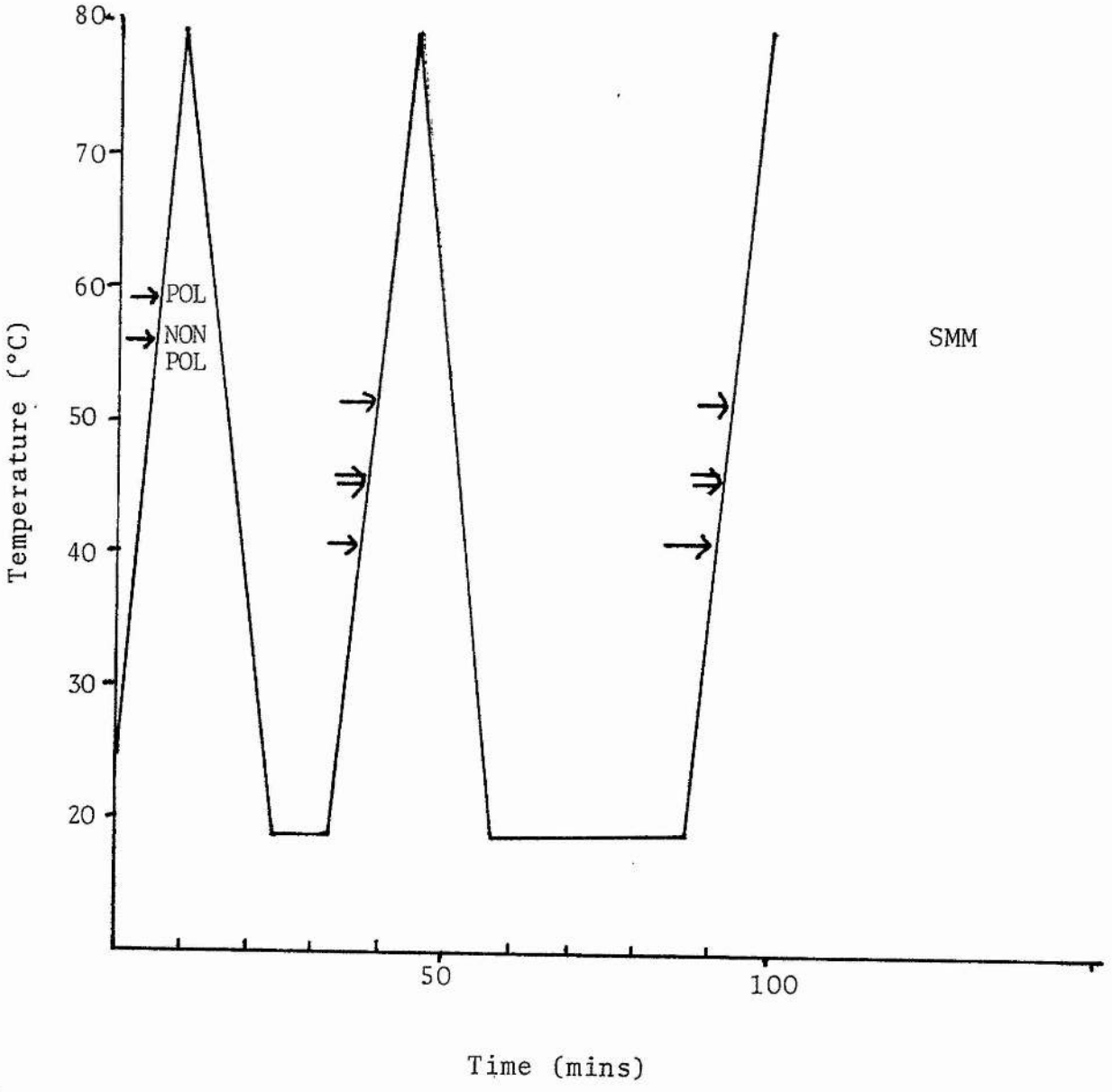
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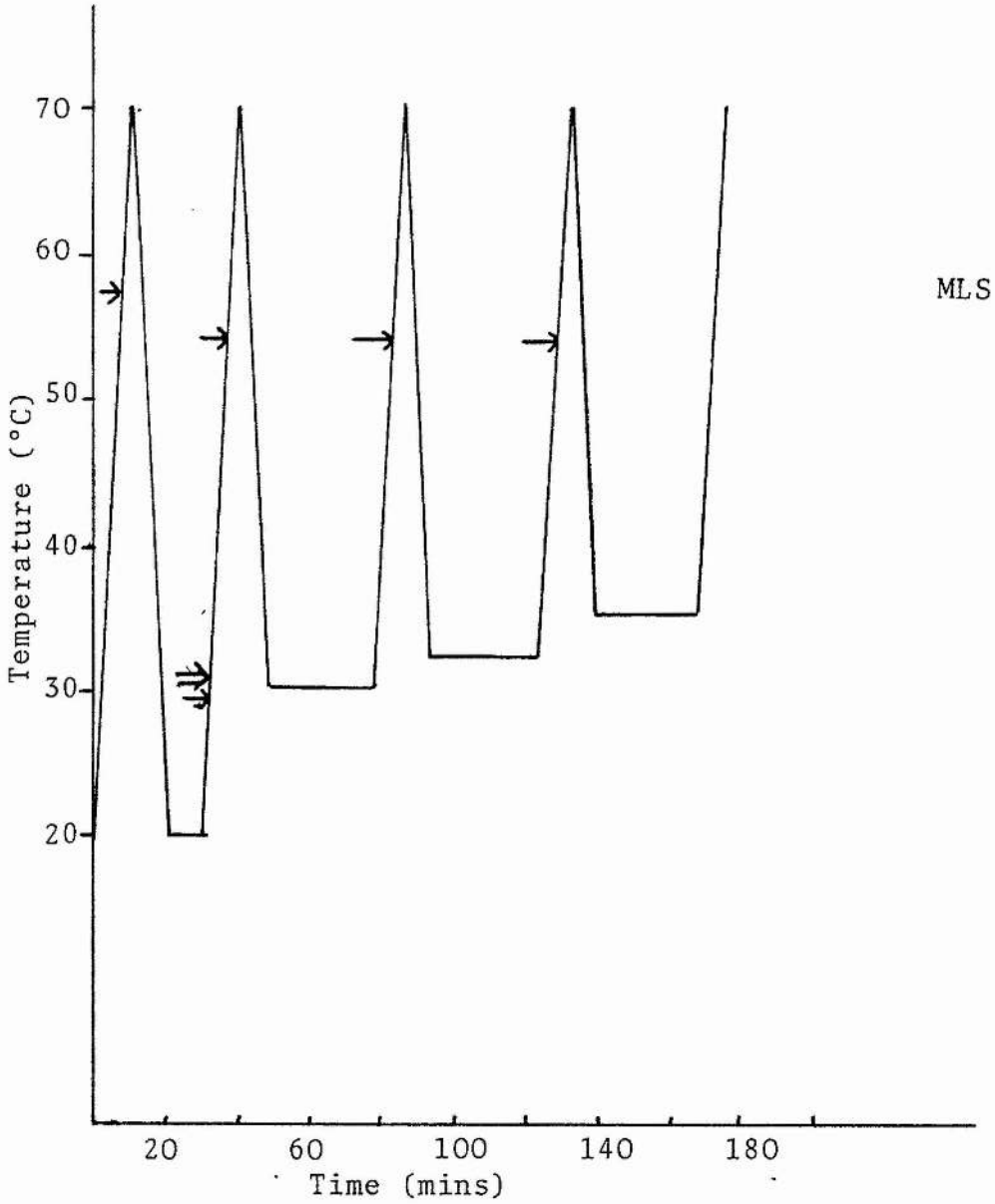
Time (mins)



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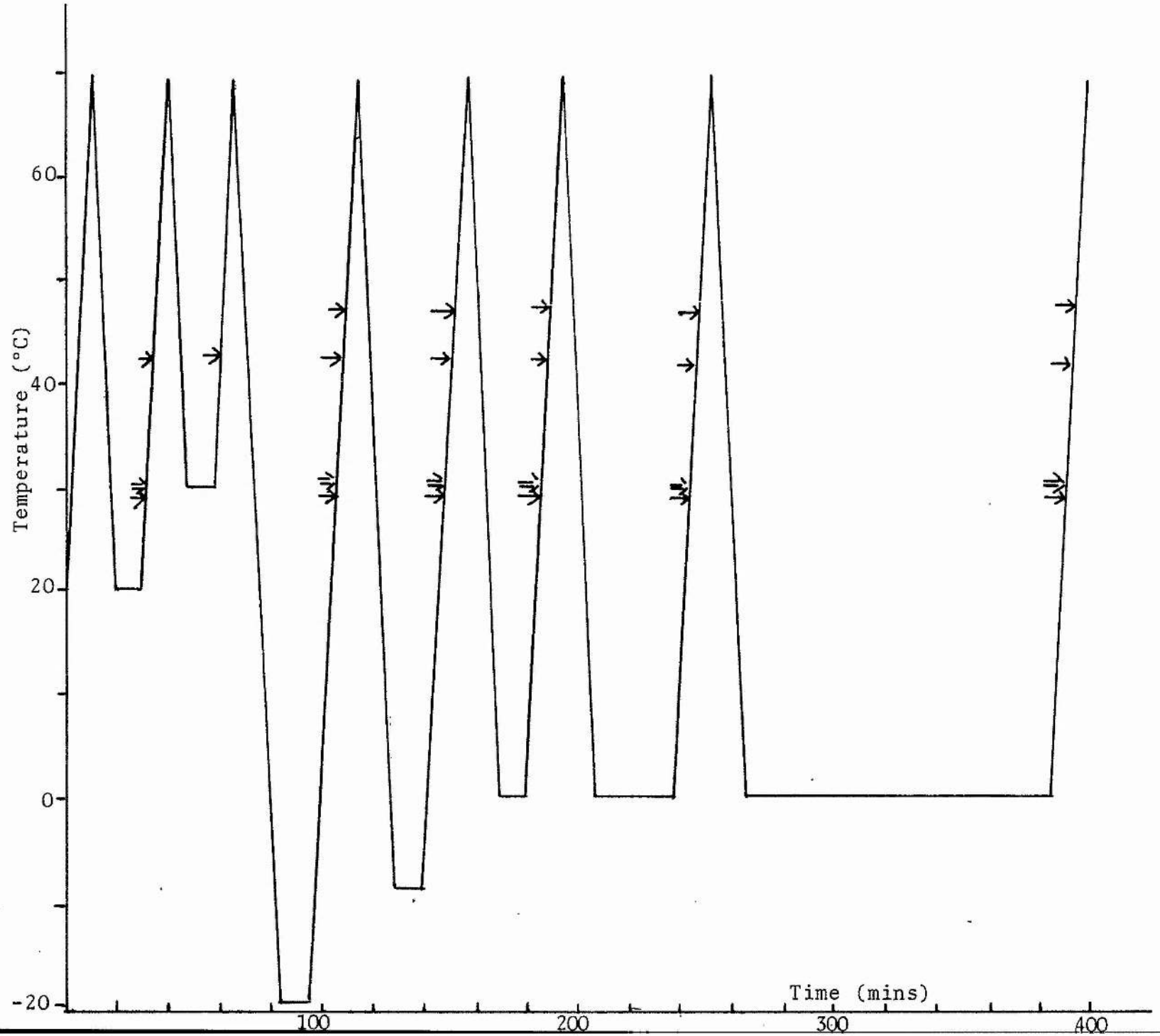

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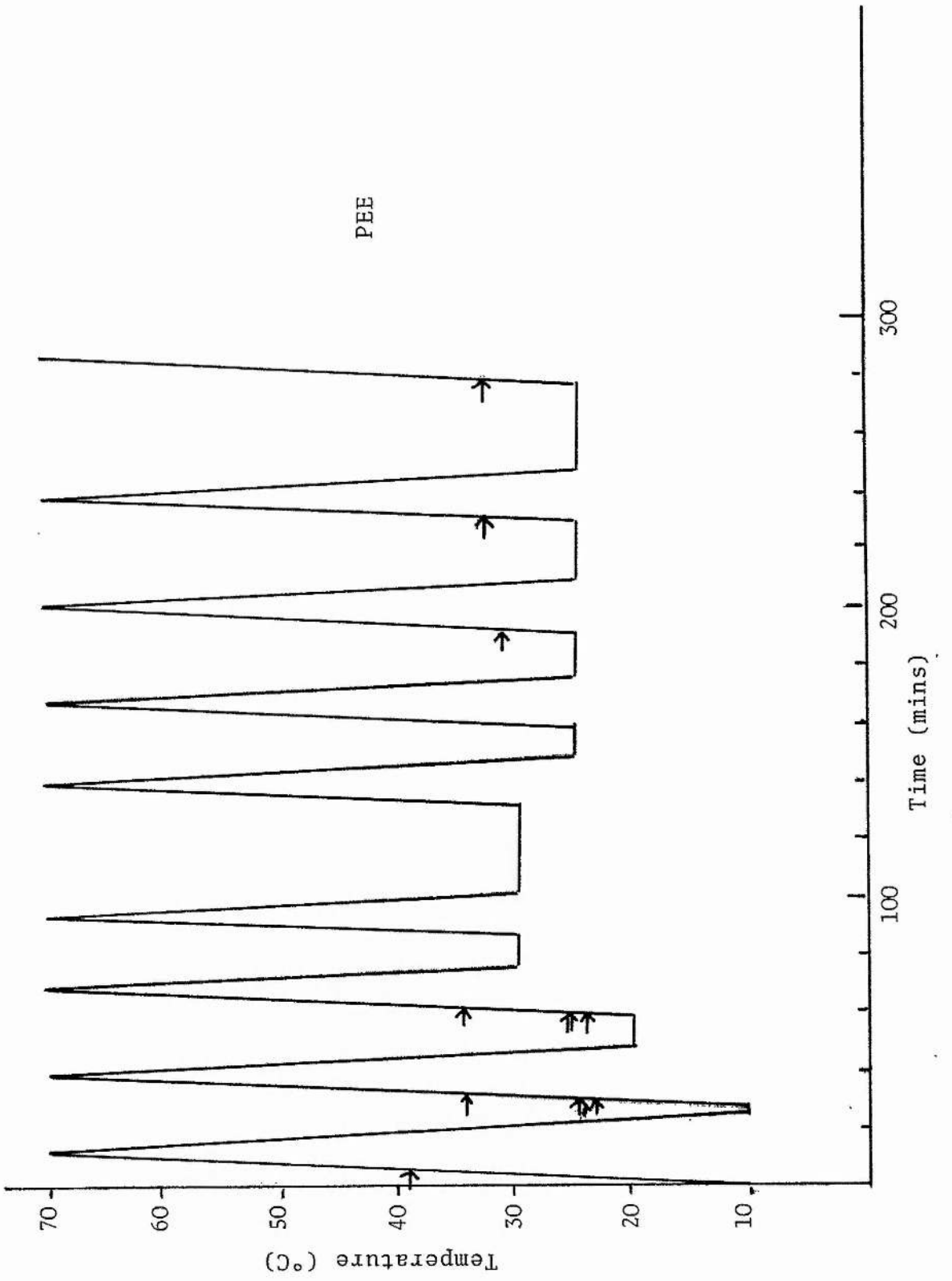
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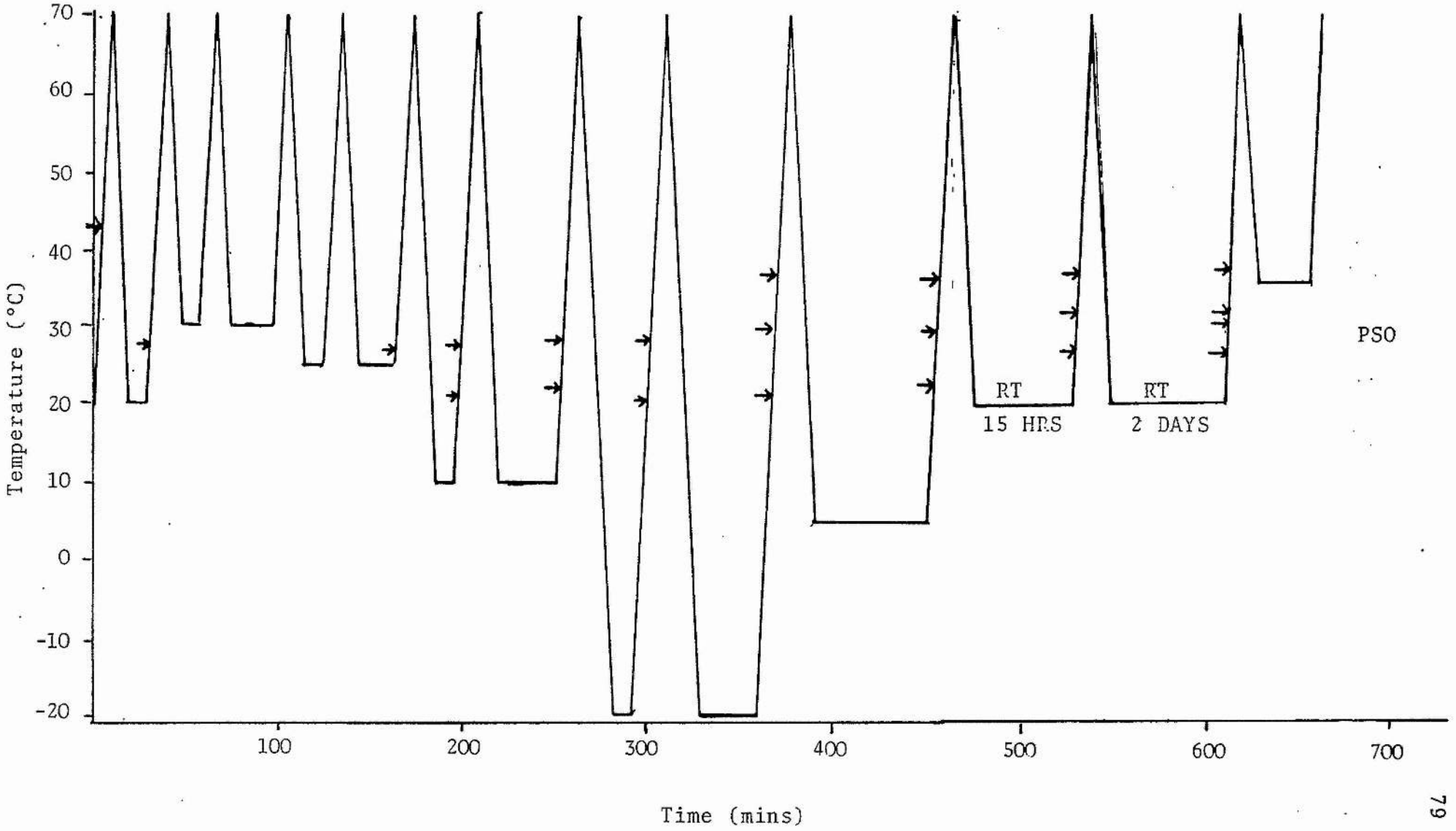
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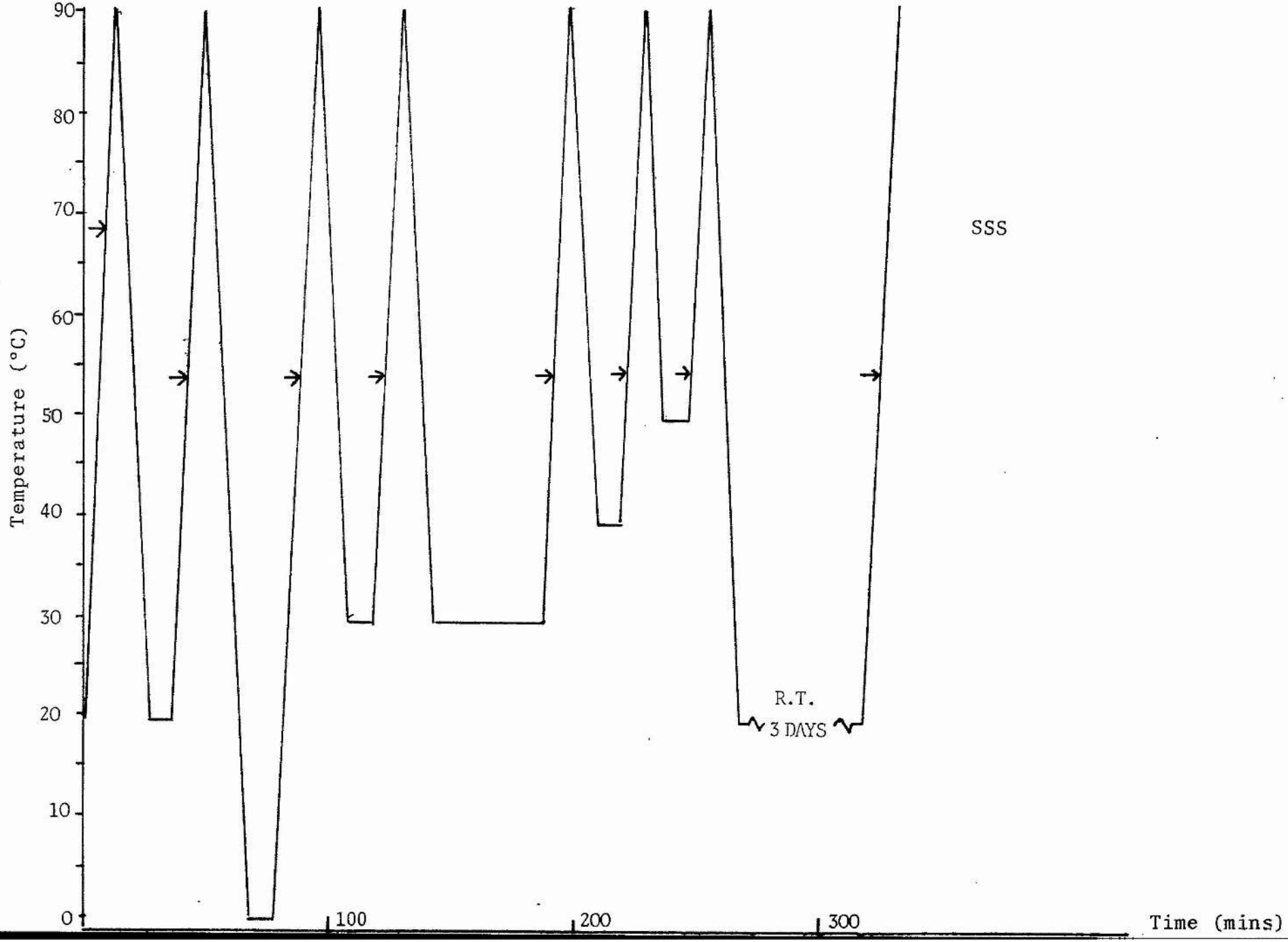
Time (mins)
RT
15 HRS
RT
2 DAYS
PSO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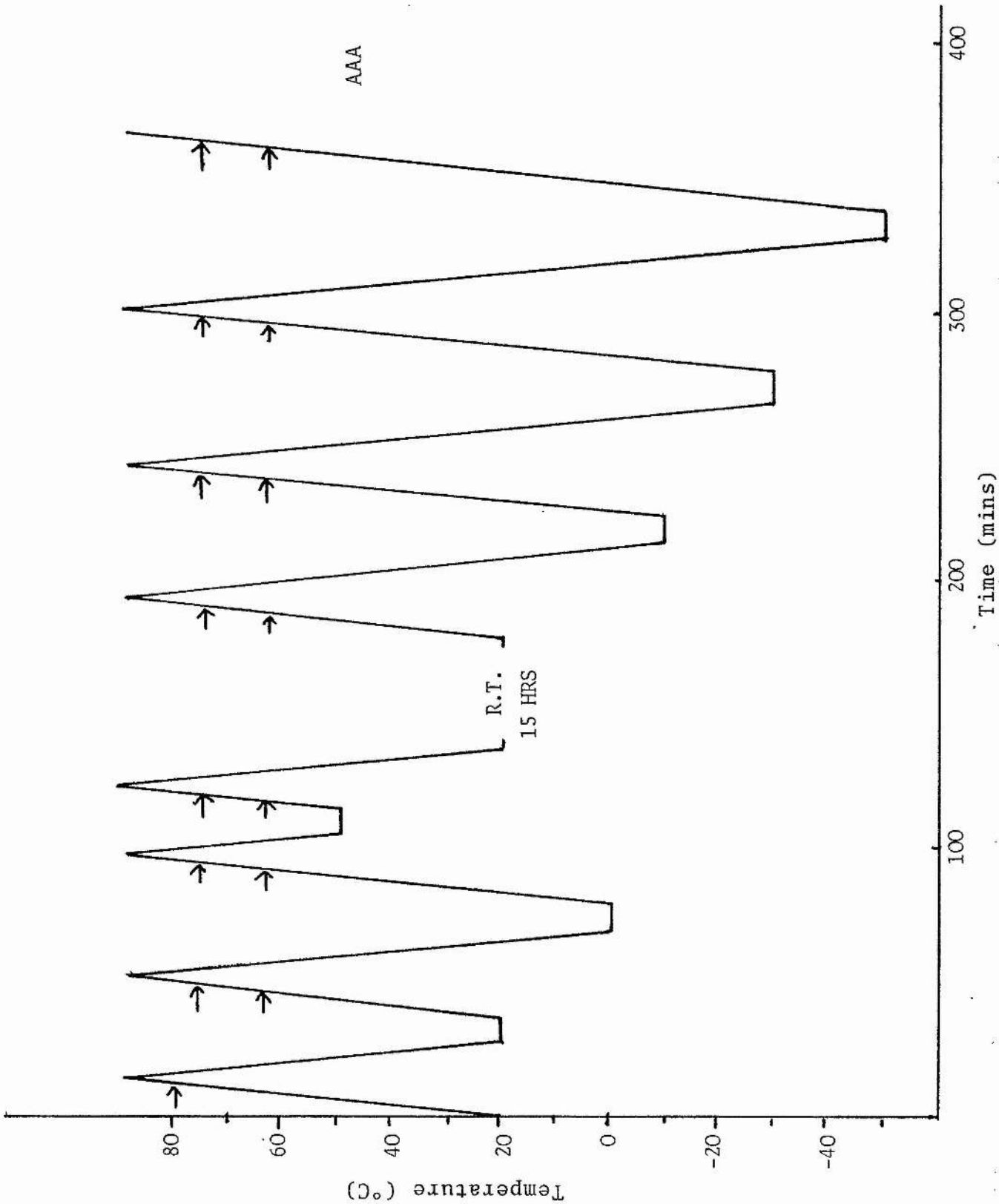
Time (mins)
SSS
R.T.
3 DAYS


AAA
R.T.
15 HRS
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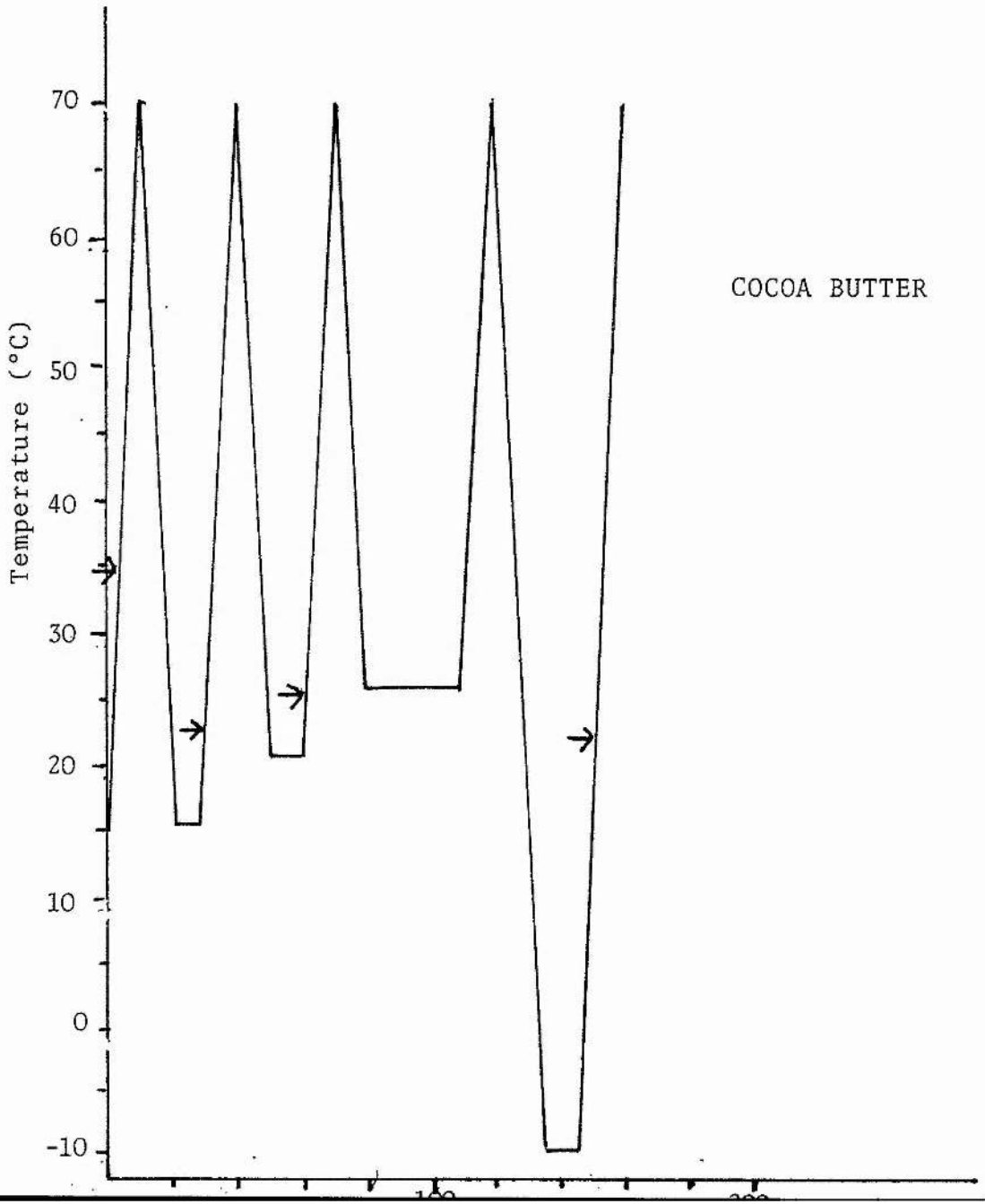
20
0
-20
-40
100
200
300
400
Time (mins)



COCOA BUTTER

Variation of Heating Rate on DSC Scan of Solvent Crystallised Material

Triacylglycerol: PEE

Size (mg)	Rate (°C/min)	m.pt. (°C)	ΔHfus exptl. J/g	ΔHfus exptl. kcal/mol	ΔHfus pub.* kcal/mol
9.2	1	38.6	152	31.05	31.5
9.0	2	39.2	151	30.85	
12.6	5	40.6	152	31.05	
11.6	10	41.8	155	31.66	

* Heating rate 8° C/min
Timms, R.E., Chemistry and Physics of Lipids (1978), 21, 113.

Triacylglycerol: LMS

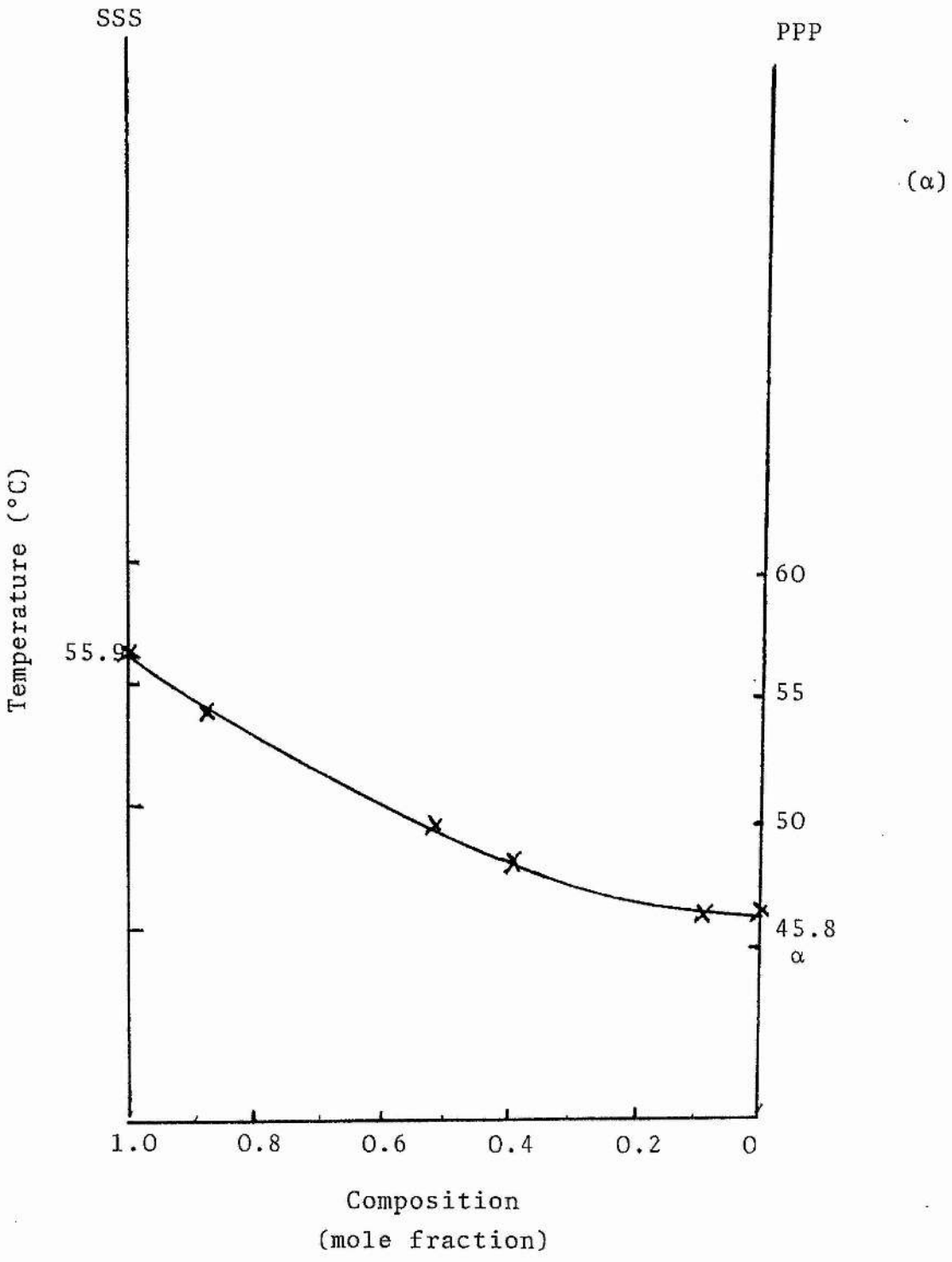
Size (mg)	Rate (°C/min)	m.pt. (°C)	ΔHfus exptl. J/g	ΔHfus exptl. kcal/mol	ΔHfus pub.* kcal/mol
8.1	1	50.3	92.6	17.15	-
7.5	2	50.9	82.5	15.28	
8.9	5	52.5	86.8	16.08	

7.5 RESULTS

FOR BINARY SYSTEMS

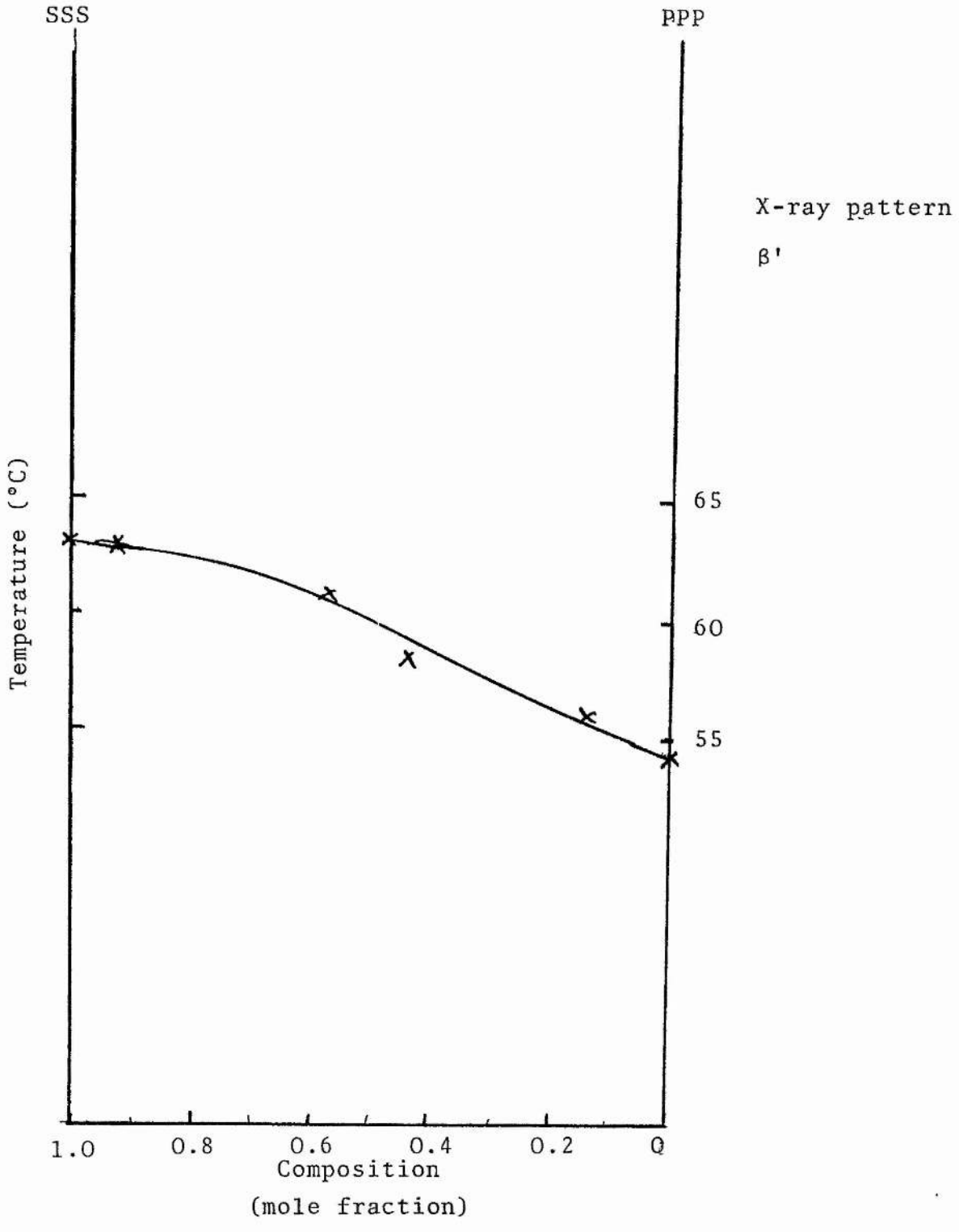
BINARY MIXTURE: SSS/PPP

CONDITIONS: Melt → 15°C 15 hours



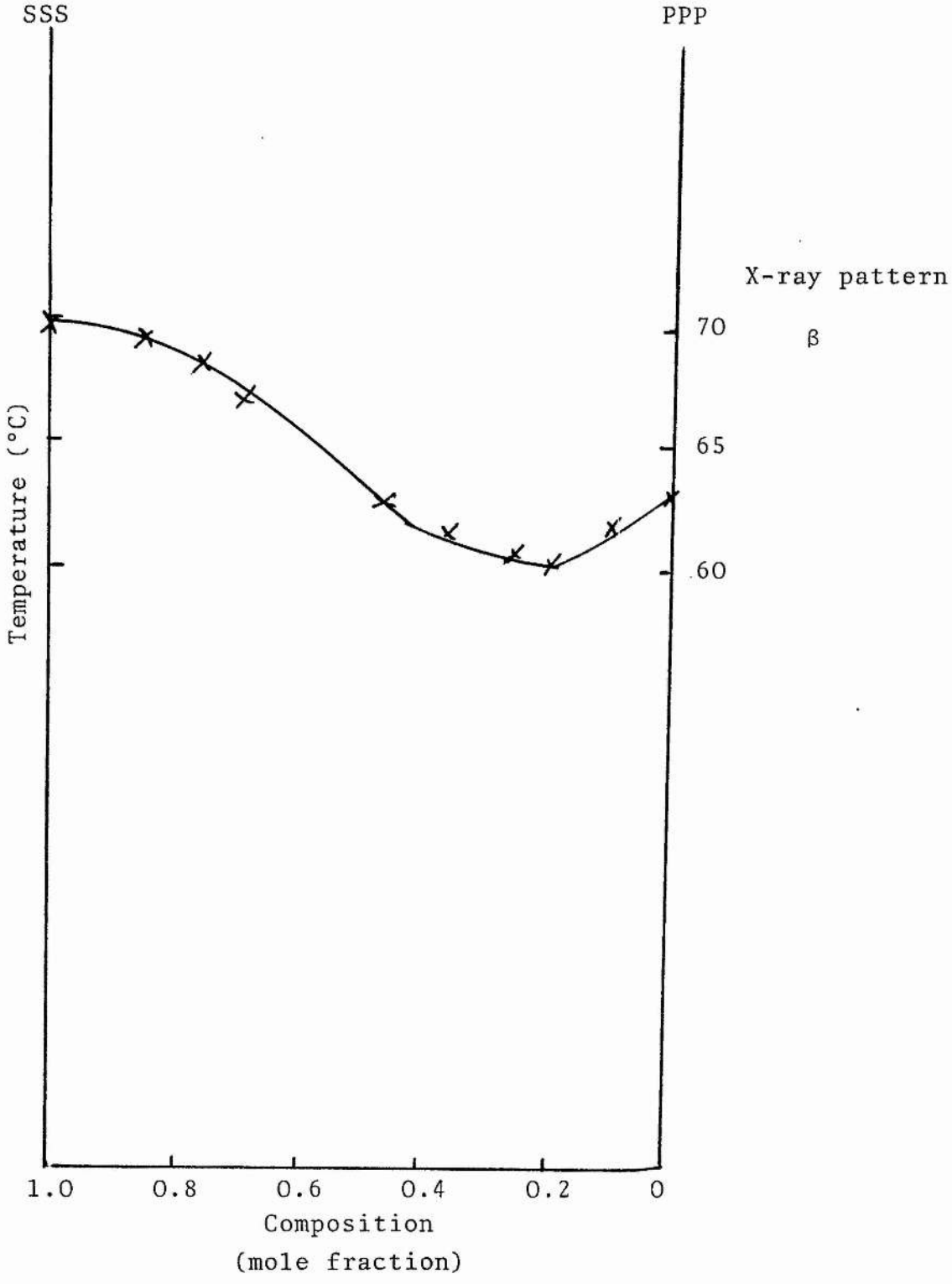
BINARY MIXTURE: SSS/PPP

CONDITIONS: Melt → 40°C 4 hours → 15°C 1 hour



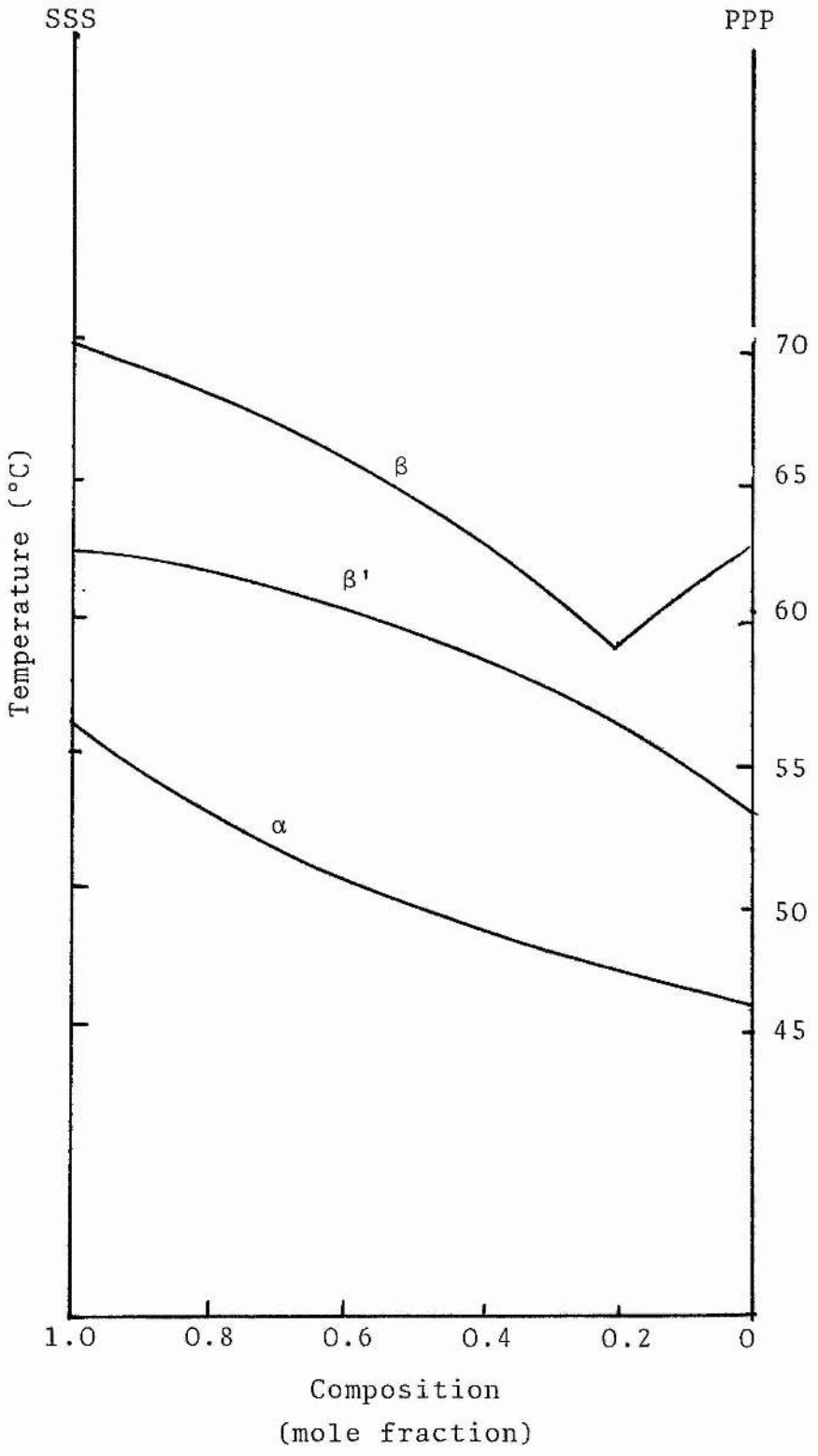
BINARY MIXTURE: SSS/PPP

CONDITIONS: Melt → 15°C 1 hour,
45°C 15 hours,
15°C 1 hour.



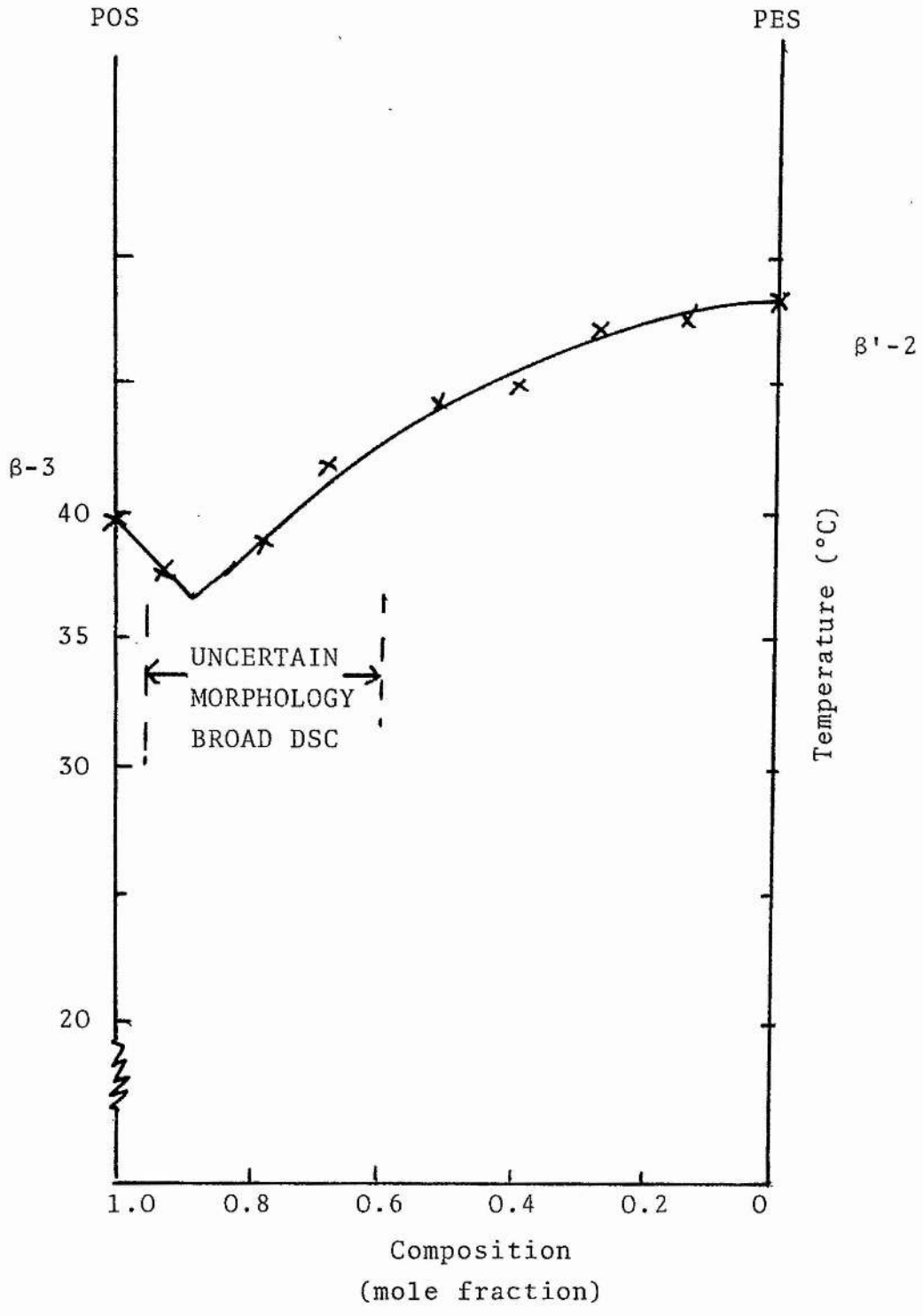
BINARY MIXTURE: SSS/PPP

SUMMARY



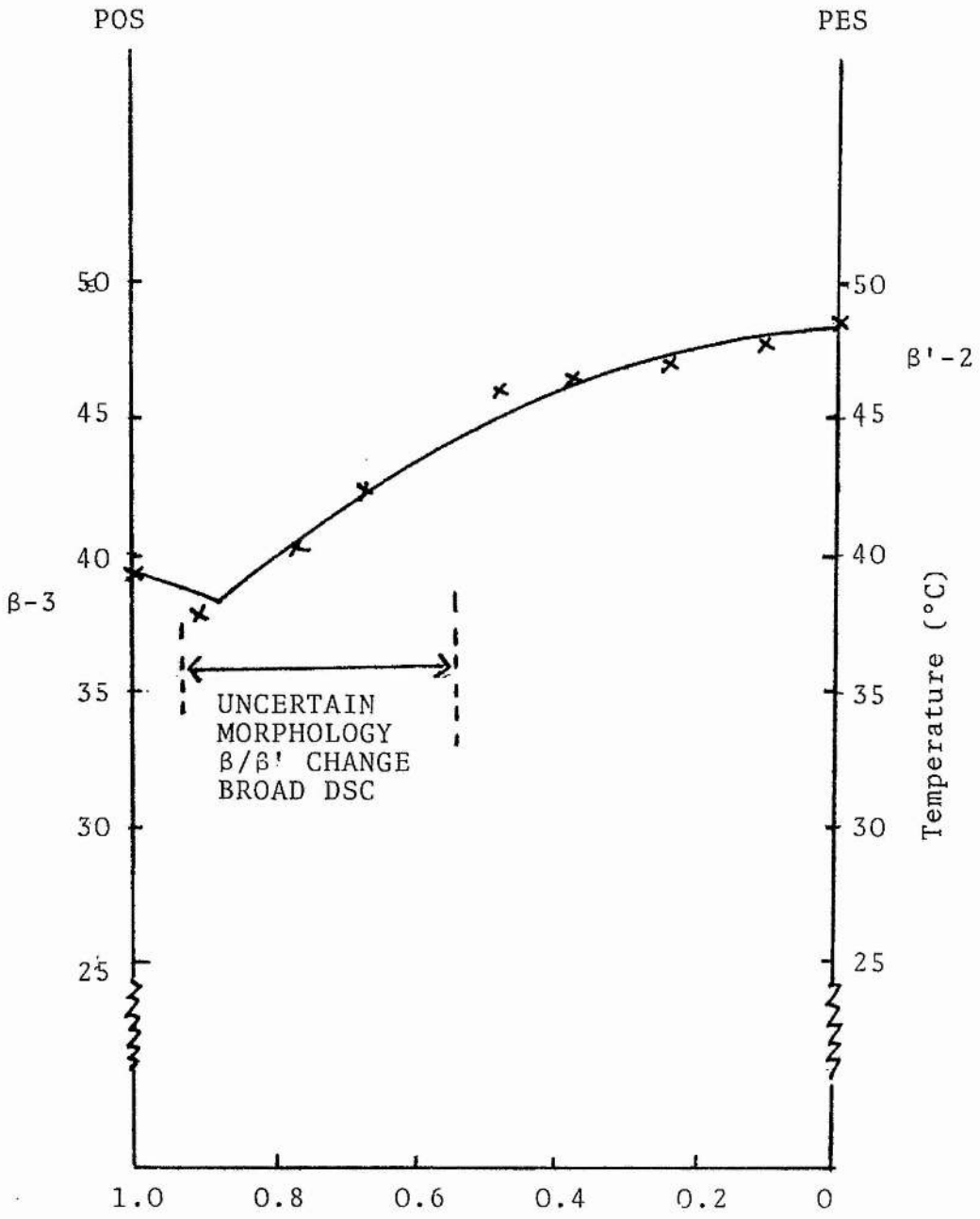
BINARY MIXTURE: POS/PÈS

CONDITIONS: Melt → 15°C 1 hour → 30°C 15 hours ≻ 15°C 1 hour



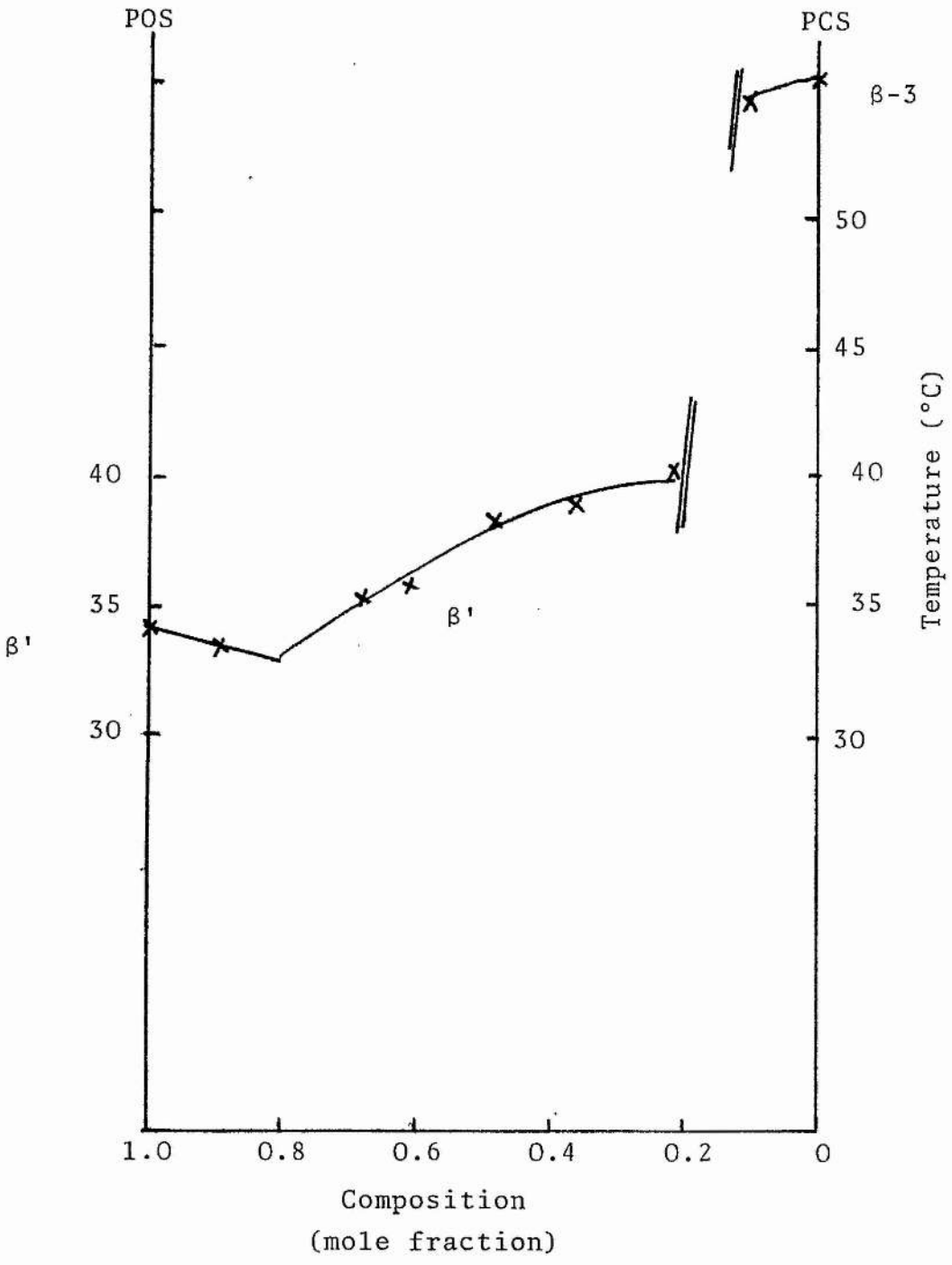
BINARY MIXTURE: POS/PES

CONDITIONS: Melt → 15°C 1 hour → 30°C 6 days → 15°C 1 hour



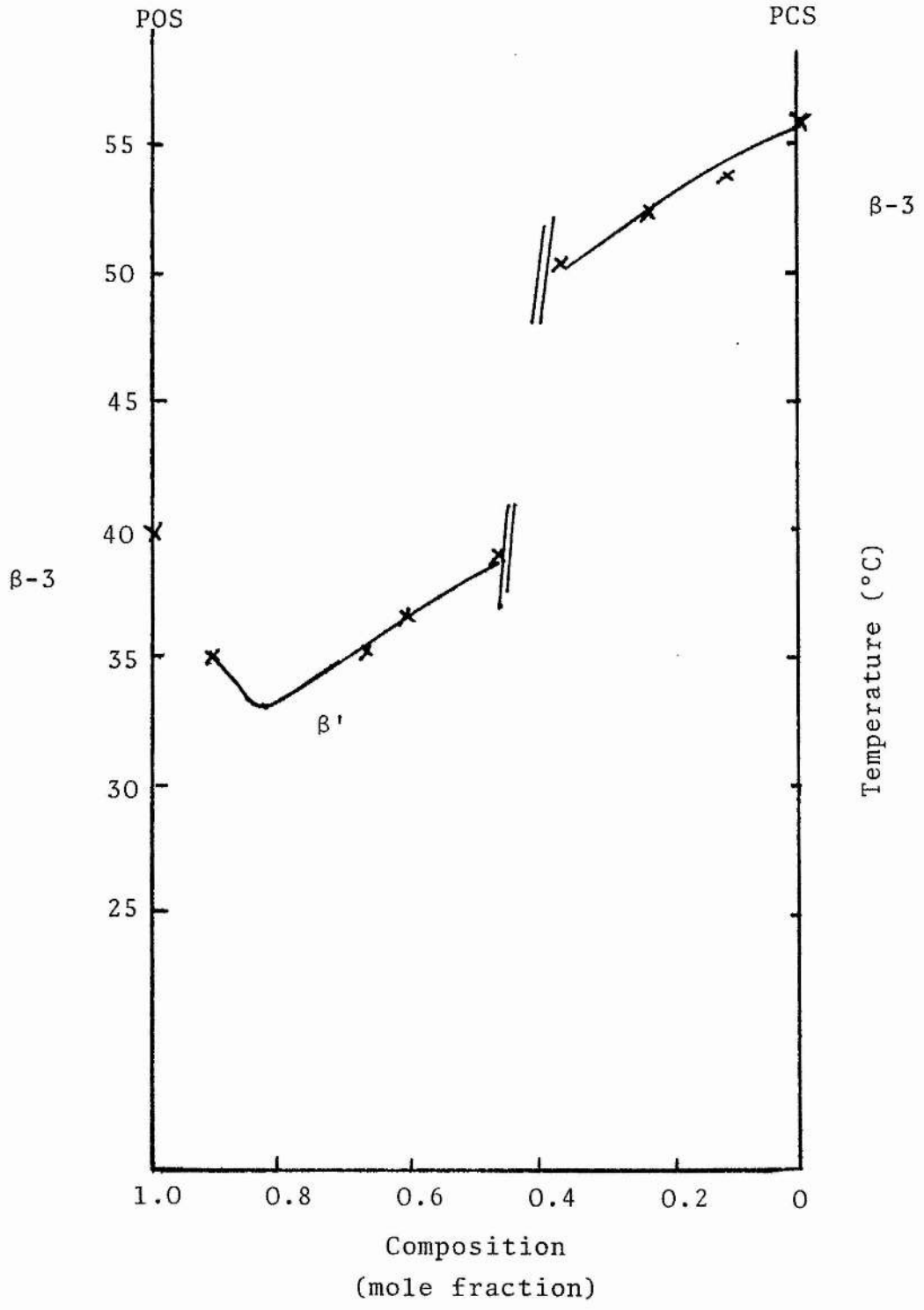
BINARY MIXTURE: POS/PCS

CONDITIONS: Melt → 15 hours 15°C



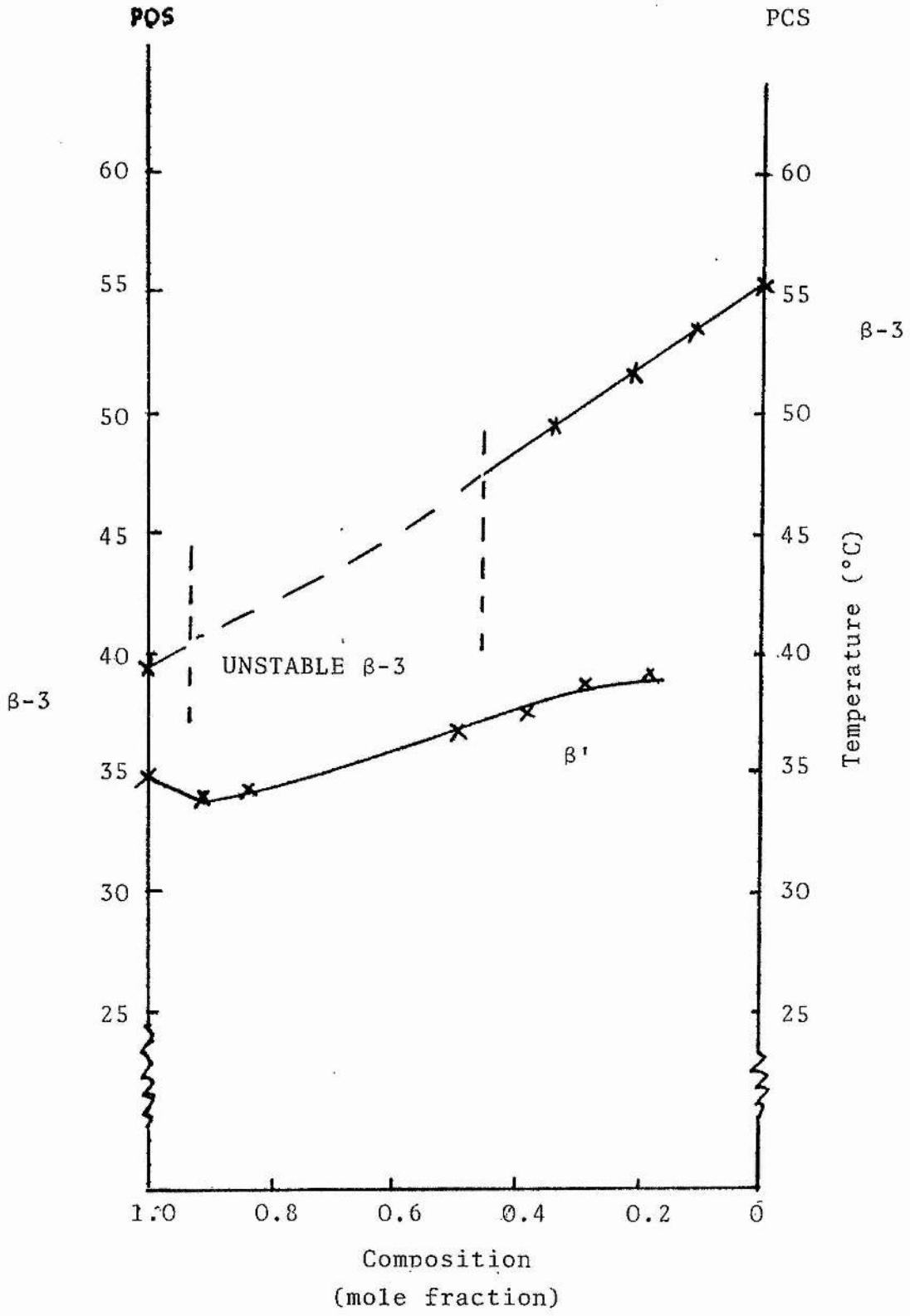
BINARY MIXTURE: POS/PCS

CONDITIONS: Melt → 15°C 1 hour → 30°C 15 hours → 15°C 1 hour



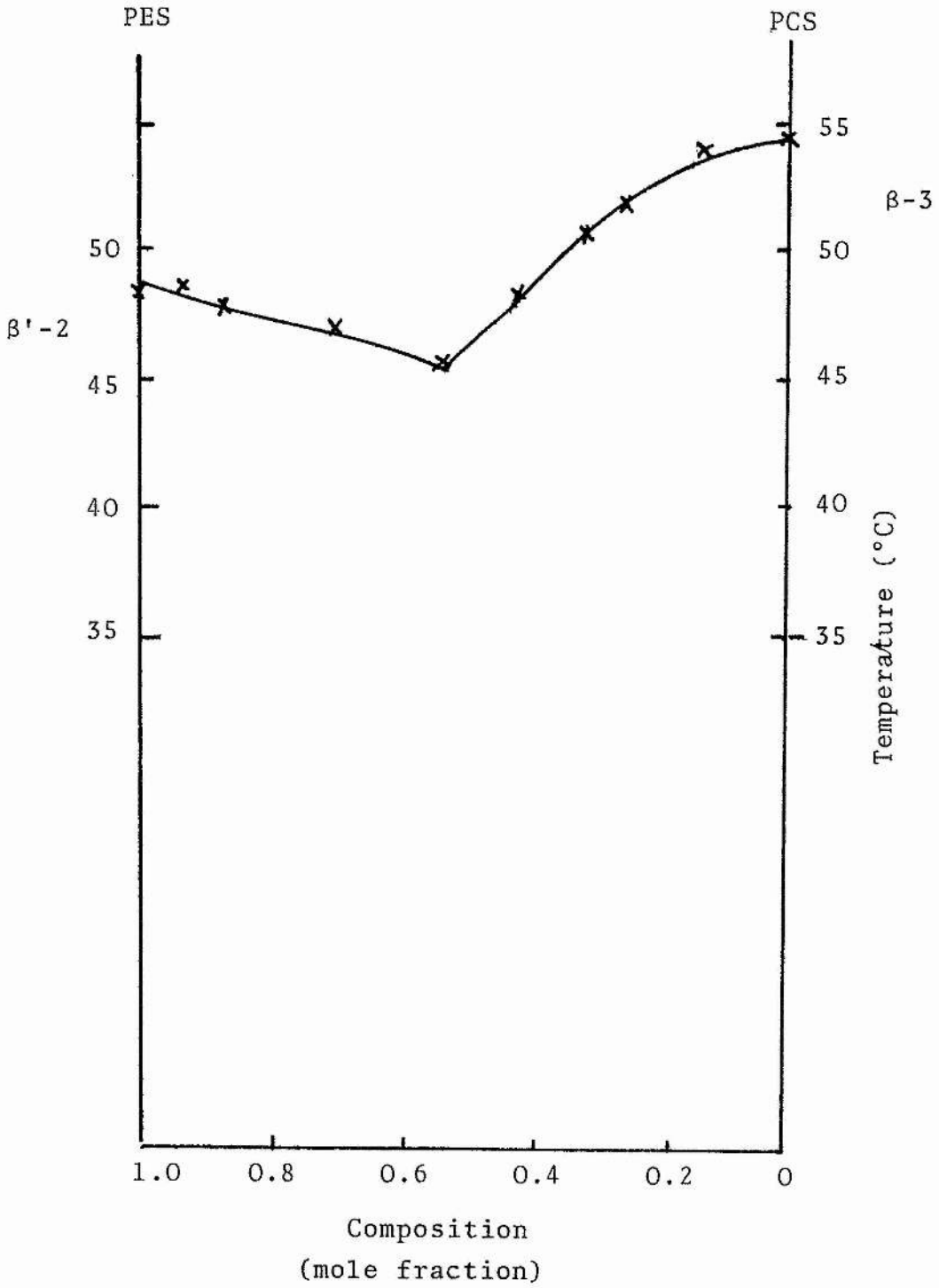
BINARY MIXTURE: POS/PCS

SUMMARY



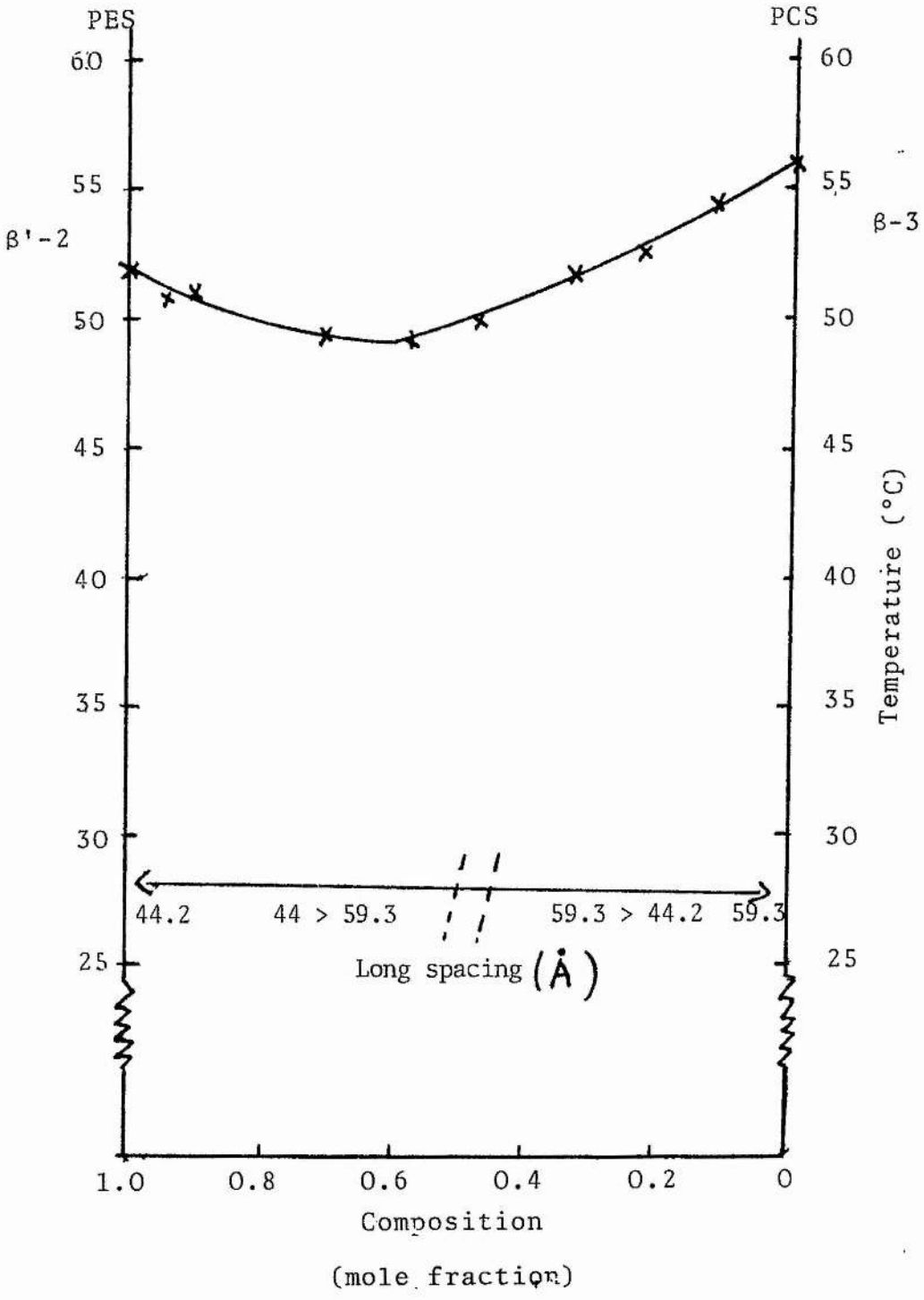
BINARY MIXTURE: PES/PCS

CONDITIONS: Melt → 15°C 1 hour →30°C 4 days → 15°C 1 hour



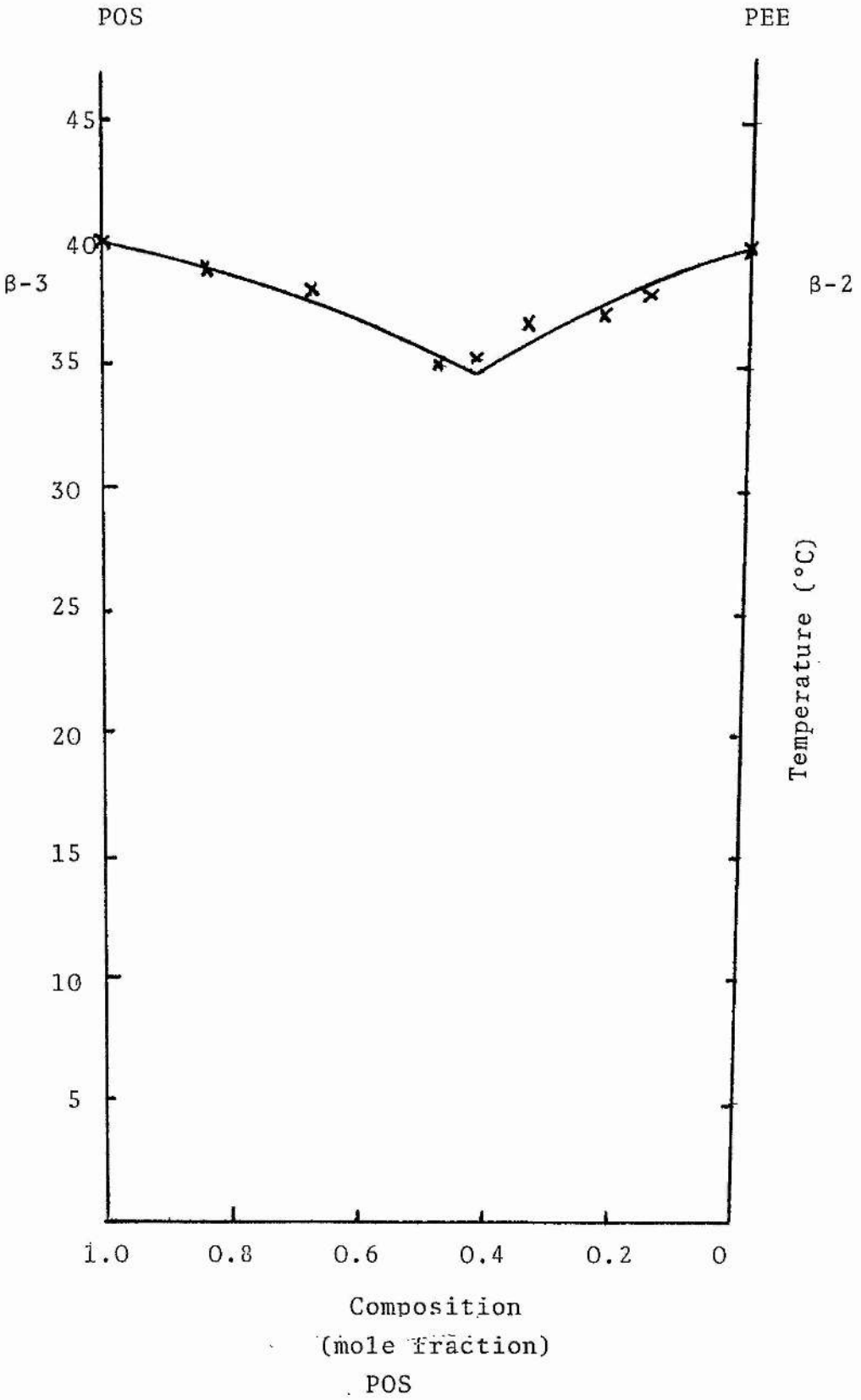
BINARY MIXTURE: PES/PCS

CONDITIONS: Melt → 15°C 1 hour → 40°C 4 days → 15°C 1 hour



BINARY MIXTURE: POS/PEE

CONDITIONS: Melt → 15°C 15 hours → 30°C 7 days → 15°C 1 hour



CHAPTER 8

DISCUSSION

8.1 Single Triacylglycerols

8.1.1 POS

i) Solvent crystallised

Solvent crystallised POS had a melting point of 39.9°C and an X-ray diffraction pattern of a β-3 structure with long spacing 63.1 Å. Material crystallised from melt indicated the presence of a β-3 structure, however, β' short spacing was also evident. The melting point of this material was 35.1°C. A β' form of POS would have a lower melting point than the more stable β form and it would seem likely that the POS melt is providing a β' form which, under the conditions of X-ray examination at room temperature, undergoes transition to the β form at a rate such that both forms be observed. The DSC scan rate may then not be adequate to show both polymorphic changes with the single peak observed concealing the melting of both forms. A clearly defined crystal structure was not evident.

ii) Thermal conditioning by DSC

Two endothermic peaks were observed using differing conditions of crystallisation although both endotherms were not present on any one scan. A melting point of ~33°C could be obtained if the melt was cooled to 20°C for 30 minutes however 10 minutes at this temperature was not sufficient to produce the polymorph. No lower forms were

observed when conditioned at 20°C. An endotherm at ~26°C was obtained when melts were cooled to 12°C for 10 to 30 minutes. Without X-ray data, and with no scan showing both endotherms, it is not possible to identify the nature of these endothermic transitions. However, the lower melting form could indicate an α form which would be difficult to obtain on an X-ray pattern due to its low melting point (close to room temperature). The higher melting form could be assigned to the β' form observed on room temperature conditioning while the most stable, β, form was not being observed due to conditioning. The form could also be concealed, as speculated earlier, by the melting of the β' form.

8.1.2 PES

i) Solvent crystallised

PES crystallised from solvent had a melting point of 53.9°C and a β-2 crystal structure of long spacing 44.2 Å. This material was examined by DSC and showed an endothermic transition at 37.0°C which was followed by an exotherm centred on 39.0°C. A further endothermic transition occurred at 48.8°C.

ii) Thermal conditioning by DSC

Melts cooled to 24°C for 10 or 30 minutes provided almost identical DSC scans to that obtained from examination of melt crystallised material described above. Thus, the crystallisation of PES into an α form which undergoes transition to a higher form (β' or β) when heated, would seem to occur in these cases.

8.1.3 PCS

i) Solvent crystallised

PCS crystallised from solvent melted at 56.6°C and had a β-3 X-ray diffraction pattern, the long spacing being 58.9 Å. Material crystallised from the melt had the same β structure and almost identical long spacing (60.9 Å). The melting point was measured as 54.5°C.

ii) Thermal conditioning by DSC

Cooling the melt at 5°C/min to 20°C and holding at that temperature for 10 to 30 minutes resulted in an endotherm at 53.5°C on reheating. No other transitions were noted indicating that PCS is particularly stable in the β-3 structure.

8.1.4 PLP

i) Solvent crystallised

Material examined directly from the synthetic method described (Part I) was observed to have two overlapping endothermic peaks when heated at 5°C/min by DSC. A sample of PLP was then recrystallised from polar and non-polar solvents (see Section 7.4,p.63 for solvent system), and after drying, were examined again by DSC. PLP crystallised from polar solvent showed only one, distinct endotherm centred on 55.8°C. A small endotherm at this temperature was also observed for material crystallised from non polar solvent, however, this was superimposed on a larger endotherm centred on 52.6°C. The X-ray diffraction patterns of both materials indicated β structures. The

long spacings were different, however, with the material crystallised from polar solvent having a 61.7 Å spacing (i.e. triple packing) and that crystallised from non polar solvent a 38.2 Å (double packing) spacing.

Material crystallised from melt gave an α X-ray pattern with long spacing 43.1 Å. A DSC scan showed an endotherm at 32.6°C for this α structure which was followed by an exotherm. Two additional endotherms at ~49°C and 54°C were observed in the same scan presumably corresponding to the two β forms of PLP, one with double and the other with triple packed structures.

ii) Thermal conditioning by DSC

Cooling a melt to 24°C for between 10 and 30 minutes resulted in two endothermic peaks and one exotherm on the reheating DSC scan. The first endotherm was centred on 32.4°C and was immediately followed by an exotherm. The second endotherm occurred at ~ 49.5°C however it was not completely symmetrical with a rather broad tail.

8.1.5 SMM

i) Solvent crystallised

The melting point of SMM taken directly from synthesis when examined by DSC showed an irregular, broad, peak. The triacylglycerol was, therefore, recrystallised from both polar and non polar solvents as done for PLP. Material crystallised from non polar solvent melted at 56.6°C while that crystallised from polar solvent melted at 59.3°C. The

X-ray diffraction pattern of these samples were both of the β type, however, non polar crystallisation gave a 41.1 Å long spacing (double packing) and polar crystallised material gave a 64.0 Å spacing (triple packing). Thus both β-2 and β-3 packing was obtained from solvent crystallisation. SMM crystallised from melt gave an α structure with long spacing 44.6 Å. A DSC scan of this sample showed an endotherm at 37.7°C. This was immediately followed by an exotherm and an endotherm centred at 47.6°C was observed. This was also followed by a small exotherm and a final endothermic transition was centred on 56.6°C. The 47.6°C endotherm could indicate a β' form as an intermediate structure between the α and the most stable β structures.

ii) Thermal conditioning by DSC

Cooling melts to 18°C for between 10 and 30 minutes produced two broad endothermic transitions which were separated by a single, broad, exotherm. The two endotherms were centred on 40.9°C and 51.4°C however both were unusually broad (~6°C at base-line). The exotherm centred ~45.5°C was irregular in shape and also broad with no return to base-line between the two endotherms. The details of the polymorphic transitions occurring under these conditions are difficult to ascertain as the nature of the thermogram indicates that information is being lost in overlapping peaks.

8.1.6 MLS

i) Solvent crystallised

Solvent crystallised MLS had a melting point of 57.3°C and gave an X-ray diffraction pattern corresponding to a β-3 structure. Material crystallised from the melt at room temperature on standing was shown to have the same β-3 form. A DSC scan of this material showed it to have a single melting point of 54.9°C. This difference of melting point between samples with the same polymorphic structure is commented on later (8.3).

ii) Thermal conditioning by DSC

Further DSC scans showed that at least two forms of MLS could be obtained. When the melt was cooled from 70°C to 20°C at 5°C/min and held at that temperature for 10 minutes, reheating at 5°C/min showed endothermic peaks at 29.4°C and 54.2°C. The latter melting point is due to the presence of MLS in the β-3 form. This could have been obtained directly, by crystallisation from the melt, however, the endothermic peak at 29.4°C was immediately followed by an exothermic peak. This indicates that the lower polymorph was crystallised from the melt and, during the heating programme, was transformed to the more stable, β-3, polymorph. Some material may also have crystallised directly to the β-3 form. The structure of the lower melting form is not clear and structure determination would require additional X-ray diffraction data.

When the melt was cooled to 32°C and held at that temperature for 30 minutes, the β-3 form (m.pt 54°C) was observed. However, after holding at 35°C for 30 minutes from the melt, no sharp melting was observed on reheating. Thus, for a 30 minute holding time and a 5°C/min scan rate, a temperature between 32°C and 35°C is the critical value for crystallisation of the β-3 form from melt.

8.1.7 LMS

i) Solvent crystallised

Solvent crystallised LMS melted at 52.6°C and had a crystal structure of the β'-2 type. X-ray diffraction data from material crystallised from the melt at room temperature showed the same crystal form however the melting point had decreased to 45.1°C.

ii) Thermal conditioning by DSC

DSC conditioning showed at least three different forms of LMS, with transition temperatures of 29.3°C, 42.5°C and 47.4°C. All three transitions could be observed after cooling the melt to 0°C or lower for at least 10 minutes. When cooled to 20°C for 10 minutes only two endothermic transitions were observed; the highest (47.4°C) being absent. This could be due to either the lack of crystallisation of this form on cooling or failure of the lower forms to undergo transformation during the heating cycle.

8.1.8 PEE

i) Solvent crystallised

The melting point of the solvent crystallised PEE was 39.2°C and the X-ray diffraction pattern indicated a β-2 crystalline form. A sample of the melt crystallised at room temperature in the β'-2 form.

ii) Thermal conditioning by DSC

Liquid samples cooled at 5°C/min to 20°C or lower gave endothermic peaks at 23.6°C and 34.6°C on reheating. Cooling to 25°C or 30°C obviously did not provide conditions for the lower melting form to be obtained. Samples kept at 30°C for 30 minutes or at 25°C for 10 minutes showed no evidence of a sharp endothermic peak. When a sample was cooled to 25°C for 15 minutes or longer however, a single endotherm was noted. This varied between 31.3°C (25°C for 15 minutes) and 32.9°C (30 minutes before reheating). The critical time for formation of a polymorph melting above 30°C, when held at 25°C, is between 10 and 15 minutes.

The melting peak at 23.6°C was followed by an exothermic peak indicating the transition to a higher melting polymorph. This higher polymorph is also formed, in the absence of the lower form, providing the conditioning at a temperature slightly above the lower melting temperature allowed sufficient time for crystallisation. The variation in the higher melting point from 31°C to 35°C could be due to differing stability of the polymorph depending on its

thermal conditioning. However a differing crystal structure cannot be ruled out without additional X-ray evidence.

8.1.9 PSO

i) Solvent crystallised

Solvent crystallised PSO had a melting point of 42.7°C and a β'-3 crystal structure. Material crystallised from melt at room temperature had an α structure and long spacing of 39.8 Å.

ii) Thermal conditioning by DSC

DSC scans showed a complex melting and crystallisation behaviour. When the melt was cooled to 20°C for 10 minutes or 25°C for 20 minutes, a single endothermic peak of ~27°C was recorded. Melts cooled to 30°C for 20 minutes or 35°C for 30 minutes showed no sharp transitions on re-heating. The 27°C endotherm was, however, observed for melt cooled to between 10°C and -20°C inclusive, for 10 minutes. In addition to this endotherm an additional endothermic peak ~21°C was observed. Extending the holding time at -20°C to 30 minutes before reheating resulted in the observation of three endotherms ~21°C, ~29°C and ~36°C. This was also the case when held at 5°C for 60 minutes. When a sample, kept overnight at room temperature (~23°C), was reheated three endotherms were also observed. However, these occurred at 27°C, 32°C and 37°C. A sample left at room temperature for two days showed four different endotherms at 27°C, 30°C, 32°C and 37°C.

The presence of three endotherms at 27°C, 30°C and 32°C in the same scan makes identification of individual forms difficult using previous data due to their small range of transition. It is possible that five forms were obtained during the conditioning regimes with melting points 21°C, 27°C, 30°C, 32°C and 37°C. The alternative to these being discrete polymorphs is that, where several similar melting points are observed in a single scan, the difference is due to the level of stabilisation of the crystalline structure. For example, a polymorph formed by transition from a lower form may give a slightly different melting point to crystals formed by direct crystallisation from the melt. This effect may only be noticed when insufficient time for stablisation of the structure has elapsed.

Throughout the heating scans no sharp exothermic peaks were observed. Any transitions between polymorphs could, however, have occurred with only partial melting of a lower form and any exothermic change would then be incorporated into the endothermic peak.

8.1.10 SSS

i) Solvent crystallised

SSS as received from Sigma had a melting point of 69.3°C and an X-ray diffraction pattern corresponding to a β-2 crystal structure. A sample of the melt left to crystallise at room temperature had an α short spacing pattern and long spacing of 51.7 Å.

ii) Thermal conditioning by DSC

SSS taken from melt to temperatures ranging from 0° to 50°C and kept at that temperature for 10 minutes or longer gave only one melting point of 55°C. This was the only endotherm (and there were no exothermic peaks) observed for melt crystallised Sigma tristearin.

8.1.11 AAA

i) Solvent crystallised

AAA as supplied had a melting point of 79.7°C and a β-2 crystal structure.

ii) Thermal conditioning by DSC

Two endothermic changes were observed during a series of DSC scans. Endotherms at both 63.7°C and 75.5°C were observed in all scans where the temperature was lowered from 90°C to between +50°C and -50°C and held at that temperature for at least 10 minutes. A sample held at room temperature overnight also showed only the two endotherms.

8.1.12 Cocoa Butter

A sample of cocoa butter stored at 15°C for several days had a melting point of 33.9°C. Further DSC scans showed at least two different forms. On cooling the sample from 70°C to 15°C and holding at that level for 10 minutes, an endotherm at 22.0°C was observed on reheating. When held at 20°C for 10 minutes before reheating, melting was observed at 24.7°. Holding at 25°C

for up to 30 minutes provided no evidence of an endothermic transition on reheating. An endotherm at 21.2°C was obtained by heating material which had been kept at -10°C for 10 minutes. This was assumed to be the same polymorph observed in the earlier scan, which melted at 22°C.

The melting peaks of these DSC scans were less sharp than for the pure triacylglycerols examined. On several occasions the peaks appeared to broaden to such an extent that it seemed likely that at least two peaks were overlapping. This would result in polymorphic transitions being concealed and useful information being lost. A series of DSC scans using slower heating rates could possibly have avoided this problem by allowing more time for a transition to occur and thus separate individual transition peaks.

At temperatures above 30°C of most DSC scans of cocoa butter, some variation in heat flow was observed which was not sufficiently large to distinguish as individual transitions.

8.2 Binary Mixtures

8.2.1 Glycerol tristearate/Glycerol tripalmitate

The binary mixture system of SSS/PPP has been reported frequently in studies of triacylglycerol phase behaviour[44] . Due to the availability of these materials and the relatively simple nature of the composition (by virtue of chain similarities, i.e. two

trisaturated, monoacid triacylglycerols with similar chain lengths) this system was studied in some detail.

The conditions required to provide the three polymorphs (α, β' and β) are given for each form on separate diagrams in the results (7.5). These results have also been summarised on one diagram for convenience of reference. This does not mean that all polymorphic forms were obtained at one time for any given mixture. The conditions for each form were often very different. Phase diagrams are strictly confined to equilibrium mixtures in the most stable polymorph. The combination of data into this type of diagram is, however, common practice and allows easier comparison with literature data[44] (see Appendix 5).

The diagram obtained for this binary mixture showed good agreement in shape of melting curves for all the polymorphic modifications. The comparison with exact 'melting' points should be avoided in this case due to differing methods of measurement and different purity levels of material. The unstable α and β' forms showed curved lines with no evidence of minimum melting points below those of single components in the same modification, i.e. no eutectic behaviour was observed. The most stable form, however, did produce a eutectic curve reaching its lowest point around 20% glycerol tristearate composition. This, again, gives good agreement with published data where eutectic points have been quoted between 16 and 25% SSS

(weight percentages). The methods used for conditioning for this system were as given earlier (7.3) and were therefore used for the study of further binary systems.

8.2.2 POS/PES

Thermal conditioning of pure components POS and PES provided the stable forms (β-3 for POS and β'-2 for PES). The β form of PES was not obtained by conditioning although this form had been observed for solvent crystallised material. The study of this system was, therefore, complicated by the components being substantially different in their crystallisation behaviour in both long and short spacing packing arrangements.

The predominant polymorphic form observed during conditioning was the β' packing imposed by the PES. This polymorph was the only form observed for compositions down to a ~30% PES composition. Between 30% PES and pure POS, a complex 'cross-over' between the β' and β forms occurred which caused difficulty in assigning a distinct polymorph to a given melting point. It proved difficult to obtain, unambiguously, a β form for compositions away from pure POS. This would indicate that the β' stable PES exerts a destabilising effect on the β form of POS. The nature of this effect makes the observation of an eutectic difficult due to the lower melting point of β' POS lying just below the β form. A smooth β' POS - β' PES line can be obtained however this does not represent the true stable forms of the system's mixtures.

Over-all, the introduction of the *cis-trans* isomers in the form of elaidic or oleic acid residues, into the 2-position of this triacylglycerol system causes a large disruption of structural behaviour. The dominant stability of the system seems to come from the β' stable PES component, even when present in relatively small quantities.

8.2.3 POS/PCS

Both POS and PCS could be obtained, in their pure state, as β-3 polymorphic modifications. However, POS in β-3 form required carefully controlled conditioning as its β' form is the preferred form under incomplete conditioning. The β-3 form of PCS was found to be particularly stable, with no other form being obtained during thermal conditioning.

The observation of stabilised β forms across the entire composition range was surprisingly difficult. A complete β stable diagram was not obtained. On moving away from 100% composition of one component, a destabilising effect of the added triacylglycerol was evident. This would imply that the reasons for the individual components exhibiting a β-3 structure are not compatible with each other. The short chain 2-position group of PCS and the *cis*-unsaturated group of POS are presumably the decisive groups assisting the β form stability in the pure components but they do not complement the packing stability when mixed.

8.2.4 PES/PCS

As indicated for previous binary studies the stable forms of PES and PCS obtained by thermal conditioning were β'-2 for PES and β-3 for PCS. This was a similar situation to the POS/PES system except that in this case the β-3 (PCS) structure has a higher melting point and greater intrinsic stability than the similar structure of POS. Thus the ability of a different polymorphic modification of a component to disrupt its stability would be expected to be less evident. The observed phase diagram indicates a distinct eutectic at around 55% (mole %) PES. This occurs at the same composition as the β'/β X-ray patterns for the samples change in prominence. Although single DSC endotherms were observable, the X-ray patterns implied the presence of both β' and β polymorphs over compositions containing more than ~10% of the 2nd component. The intensities of the X-ray patterns did, as mentioned, change smoothly over the composition range. The same effect was noted for long spacing, again the intensity change between double and triple packing occurring around the eutectic. One individual component polymorph does not seem, in this case, to have sufficient enhanced stability to dictate the greater part of a two component phase system such as this. The similarity of melting points of the components may have some effect on this behaviour.

8.2.5 POS/PEE

POS and PEE were both obtained in β stable polymorphic forms by thermal conditioning. The melting points of these two triacylglycerols in these structures were almost identical (~40°C). The POS β form was in triple packed modification while PEE had double packing. When studied as a binary mixture, the melting point of each structure was decreased by addition of the other component until a eutectic point was reached at ~50% composition. The decrease in melting point was ~5°C resulting in the eutectic melting point at ~35°C. The destabilisation of the POS β structure observed in some earlier mixtures was not evident in this case.

8.3 Conclusions

The correlation which is possible between the results obtained (single and binary triacylglycerol systems) and the chemical composition of the molecules involved, is limited. For direct links to be drawn between crystallisation behaviour and chemical structure, a much larger range of systems would require examination. The discussion given above does, however, indicate some of the more obvious trends for the behaviour of the systems studied. The examination of triacylglycerols in this way can provide a useful insight into certain effects where a series of compounds are of particular interest. The collection of data on similar systems is also of value for accumulating information on which to base a more theoretical approach. The use of computer simulations on triacylglycerols has recently developed[35,60]. The area of combining

theoretical modelling techniques with phase studies appears to hold interesting possibilities for the future. The limitations of examining phase systems by the techniques used currently should, however, be mentioned.

When examining the data acquired from DSC and X-ray powder diffraction experiments certain problems were encountered which will be more fully discussed below.

One of the problems in the interpretation of DSC data is caused by the overlapping of endothermic peaks where two polymorphs undergo transitions at similar temperatures. A possible solution to this would be the use of different heating rates in order to allow transitions to occur at their own rate and not be forced excessively by rapid heating. Faster heating rates were also noted to record higher transition temperatures for the same change.

It was noted that samples of material with the same X-ray diffraction pattern did not always show the same melting/transition point when examined by DSC. This was particularly true of solvent crystallised material compared to thermally conditioned samples. Although samples of a pure compound in a known crystalline form might be expected to show the same melting behaviour, it has been reported[54] that there may be a spread of melting point depending on the degree of stability of a crystalline sample. This effect is of particular importance when dealing with binary systems where the stabilisation level of a sample is of particular importance.

X-ray diffraction

During X-ray powder diffraction examination of some pure triacylglycerols, as well as binary system samples, some

difficulty in assigning short spacing patterns to an unambiguous structure was encountered. This could be due to the presence of mixtures of two or more crystal structures. Where this was the case for binary systems, both forms were indicated. When this occurred for single triacylglycerols a study of X-ray pattern with time i.e. a more controlled stabilising method would have been of benefit. An additional benefit, which would have made correlation between DSC thermograms and X-ray data more direct, would be the use of temperature programmed X-ray diffraction. By this technique the change in X-ray diffraction pattern with temperature (i.e. change in crystal structure) could be more directly linked to a particular transition during a DSC scan. X-ray data at temperatures < room temperature would also be advantageous.

Study of binary systems

Having obtained samples regarded as being stabilised they should then be maintained at a temperature just below their melting point for a period of weeks to ensure no further transitions occur over prolonged storage.

During this study the polymorphs obtained and their transitions could only be observed at or around room temperature due to limitations of equipment. The conditioning temperatures used were chosen to allow for eutectic formation which could lower the melting point of a given composition to give a discontinuity in the phase diagram. Where a discontinuity was observed the conditioning procedure was revised and alternative temperature regimes imposed.

During conditioning polymorphic transitions were favoured by holding samples just below (2-3°C) the melting/transition point of the lower form. If samples were held at too high a temperature (too close to the melting point) then the melting of the lower form may produce liquid of unknown composition which may then crystallise in a different form. The liquid may also remain until cooled for analysis and then crystallise to a lower polymorph. This problem may be solved, provided sufficiently large sample sizes are available, by additional analysis during conditioning by wide-line or pulsed n.m.r. The solid fat present would then be monitored when required to avoid partial melting causing false deductions being made from a series of results.

Where binary studies are performed on systems with racemic triacylglycerols it should be remembered that the racemic triacylglycerol is already, technically, a binary mixture containing two optically active isomers. Stabilisation is therefore, made that much more difficult due to the increased number of 'components'.

In conclusion, it can be said that some useful data for determination of triacylglycerol compatibility can be gained from binary mixture studies and phase/polymorphism examination. The conditions of preparation and the techniques employed for obtaining the experimental data must, however, be fully recorded for the results to have any meaning. Comparisons with other methods must be done with care.(61)

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A P P E N D I X 1

Fatty acid notation and abbreviations

Acid	Notation	Abbreviation
Capric	10:0	C
Lauric	12:0	L
Myristic	14:0	M
Palmitic	16:0	P
Stearic	18:0	S
Oleic	18:1 (9-*cis*)	O
Elaidic	18:1 (9-*trans*)	E
Arachidic	20:0	A

Table 1

A P P E N D I X 2

Urea Crystallisation

Percentage of acid to be removed by crystallisation	1-5	6-8	9-11	12-30	31-40	41-50	51-60	61-80	81-98
Amount of urea to be used in relation to weight of acid to be removed	20x	15x	12x	10x	8x	6x	5x	4x	3x

Table 2

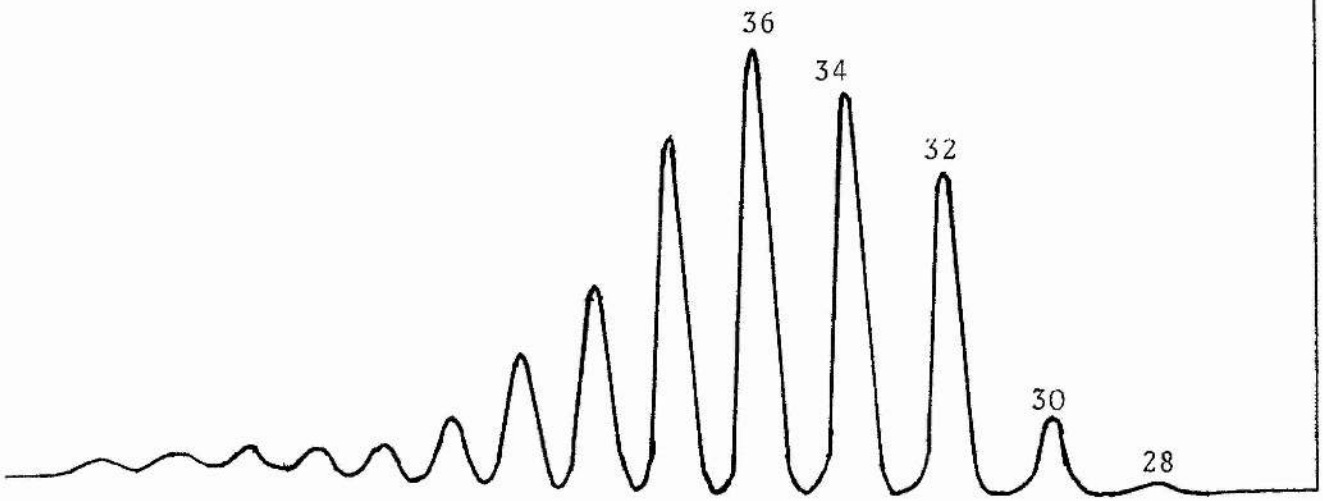
Sample:- 5% Coconut oil in chloroform

Column:- 3% OVI

Temperature:- Temp prog 4°C/min

250 → 350°C

Attenuation:- 50 x 10^2

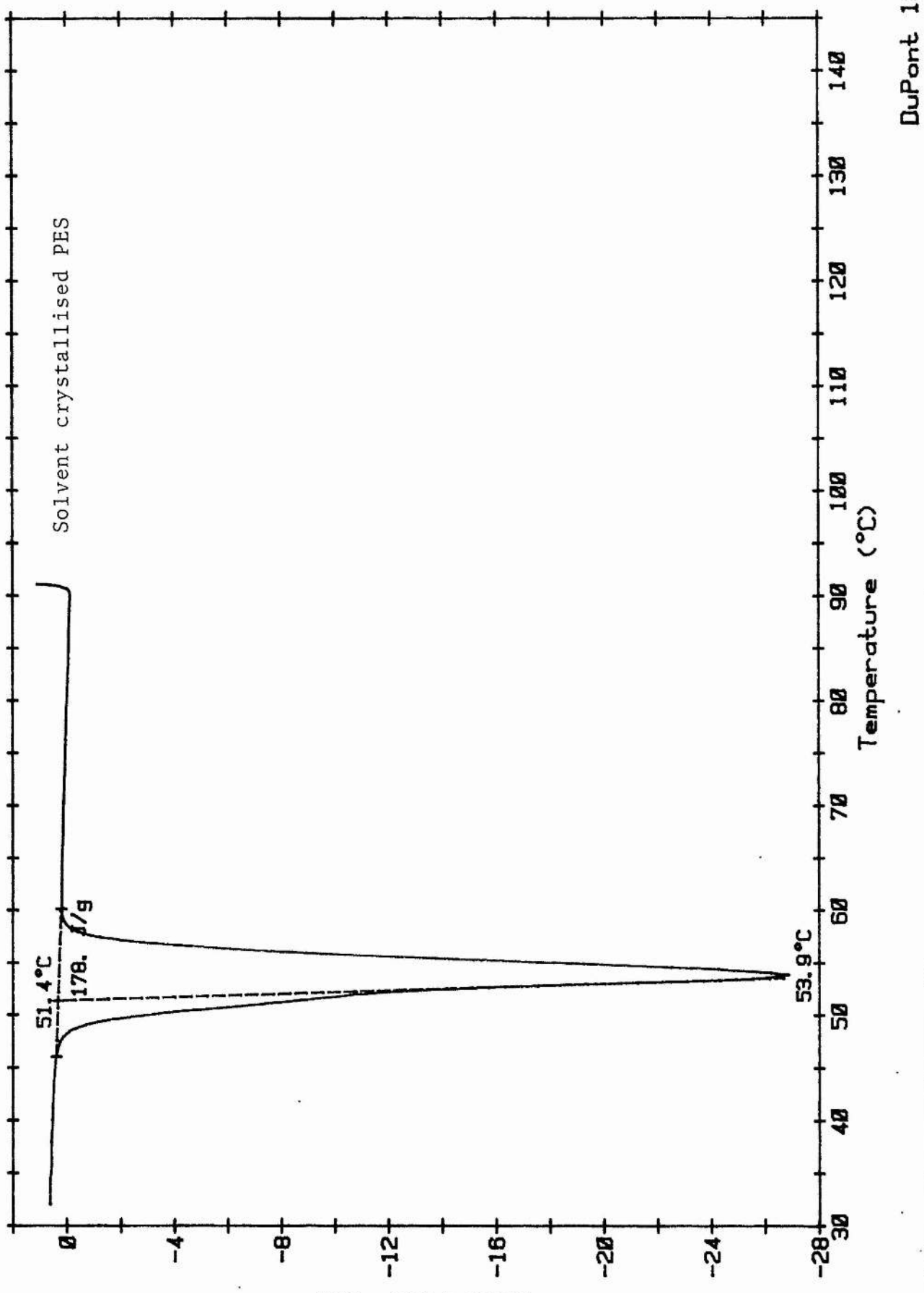


A P P E N D I X 3

Examples of DSC scans and X-ray patterns (short and long spacings).

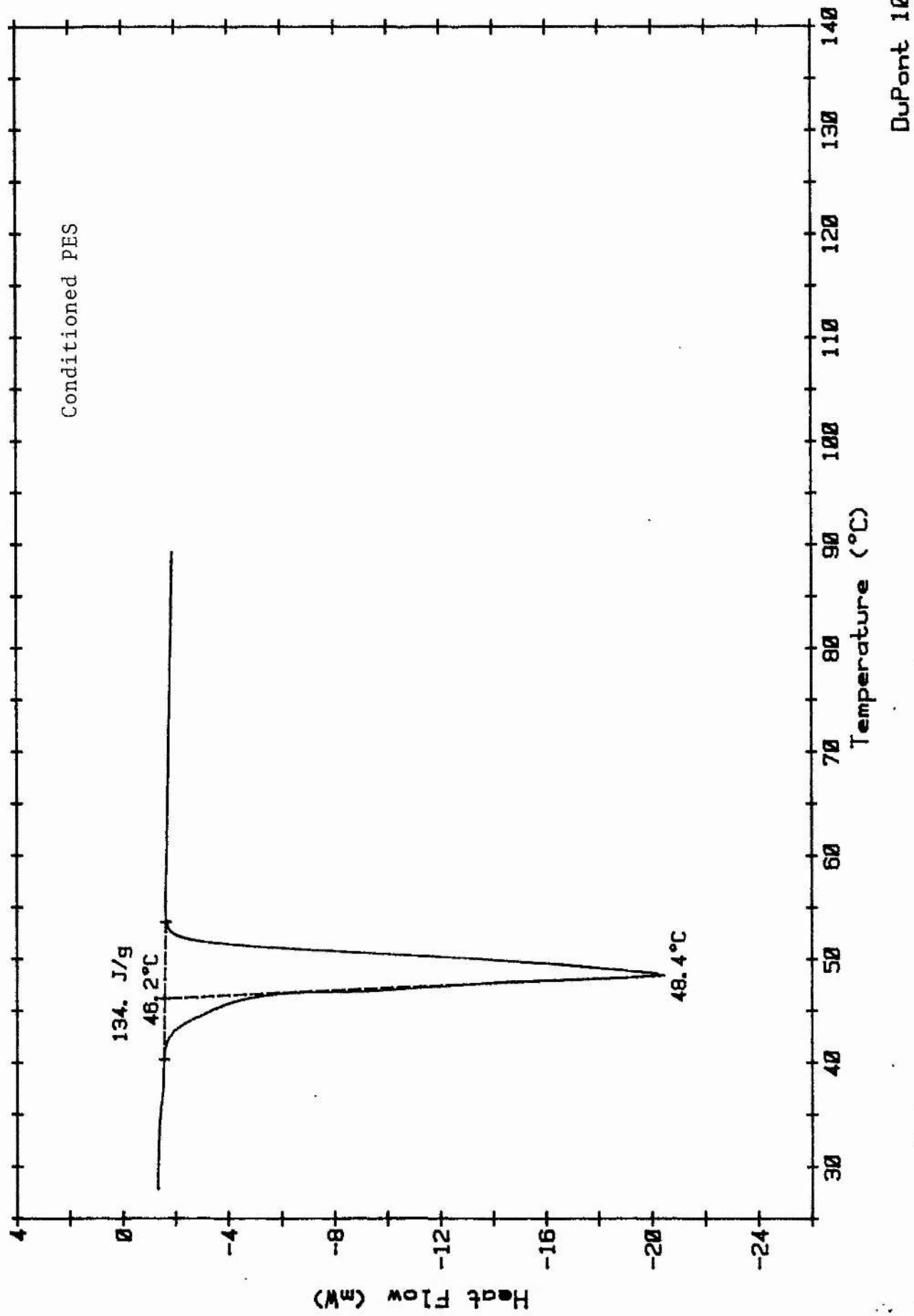
PES and PCS

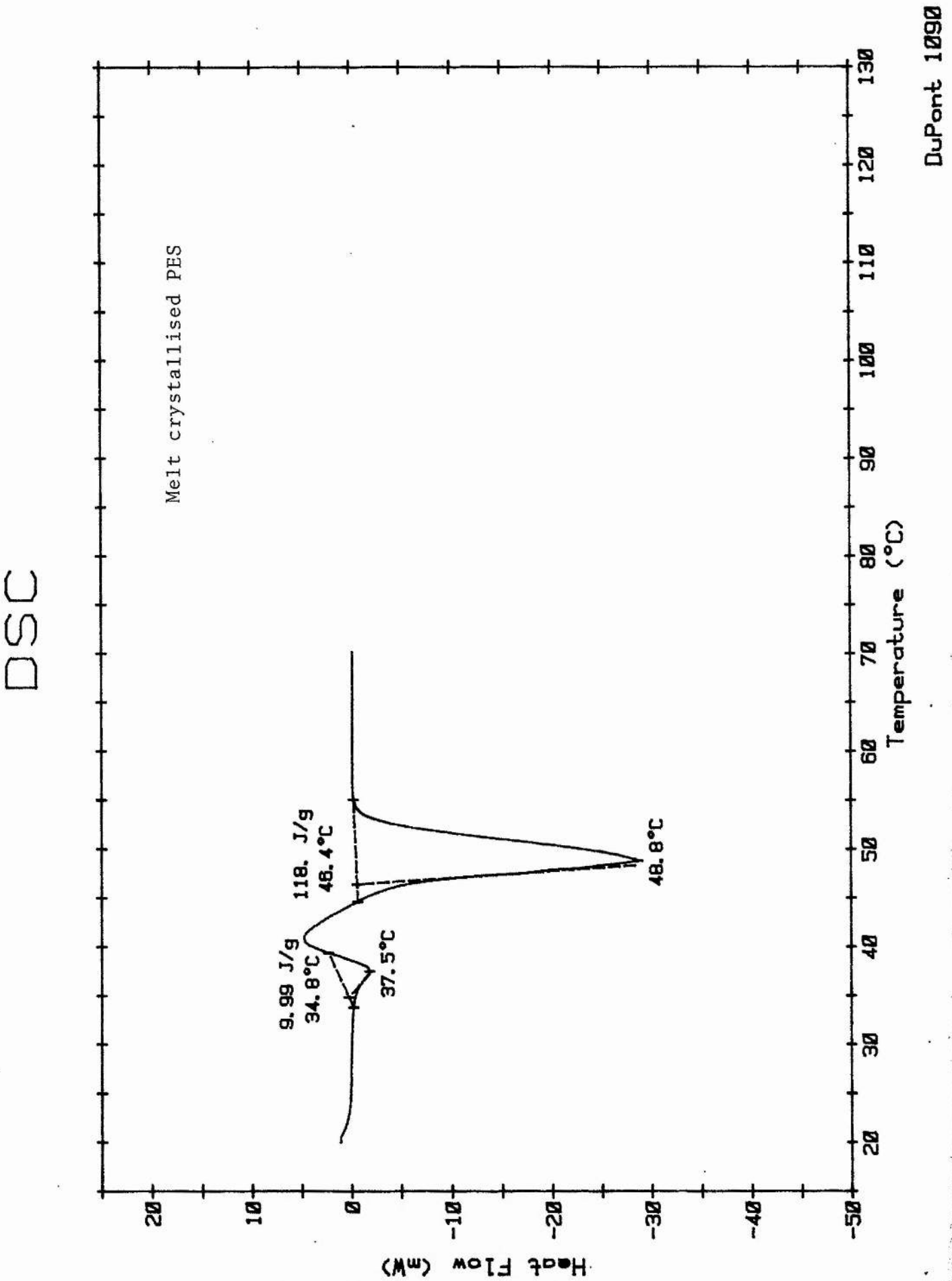
DSC



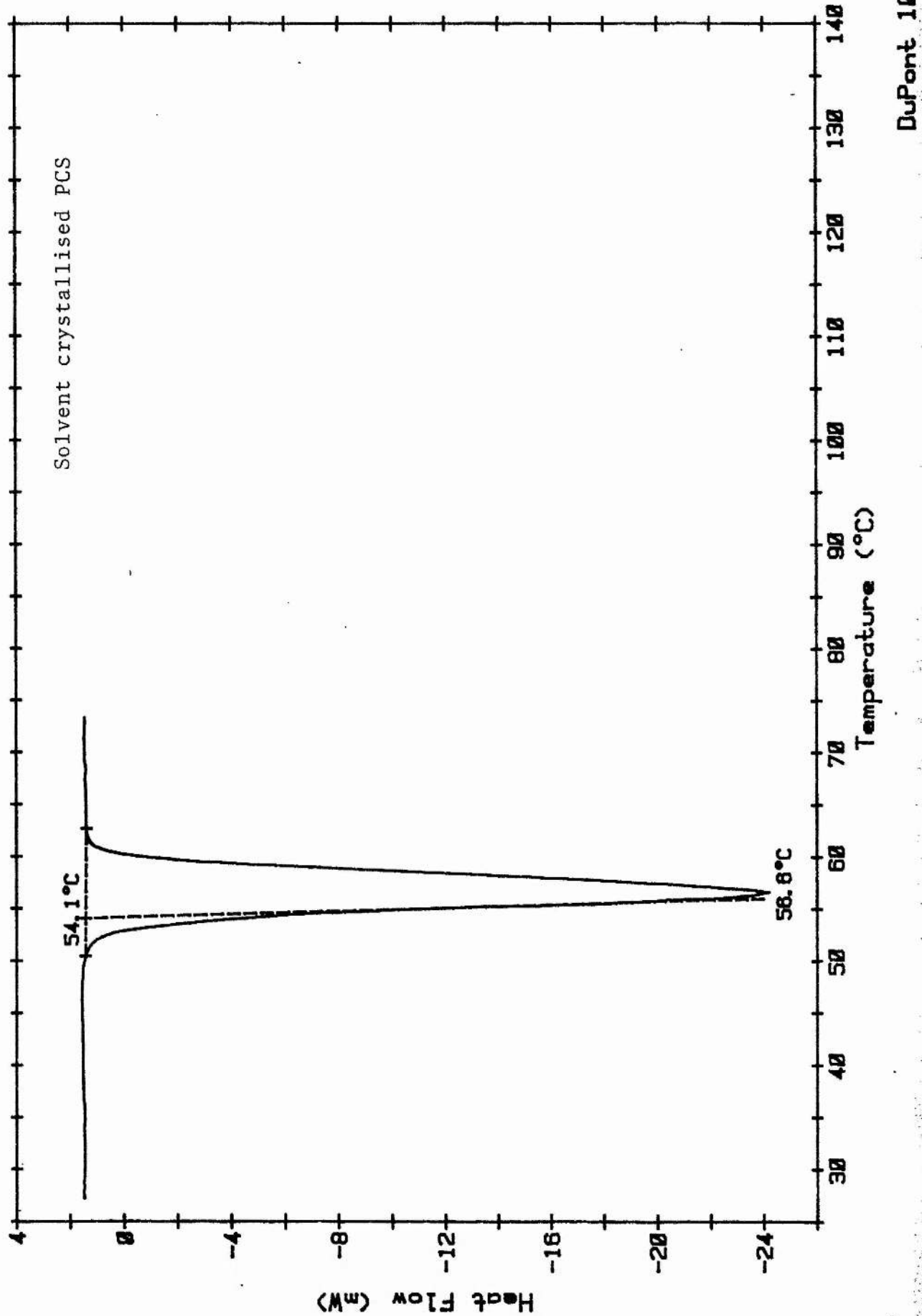
DuPont 1090

DSC

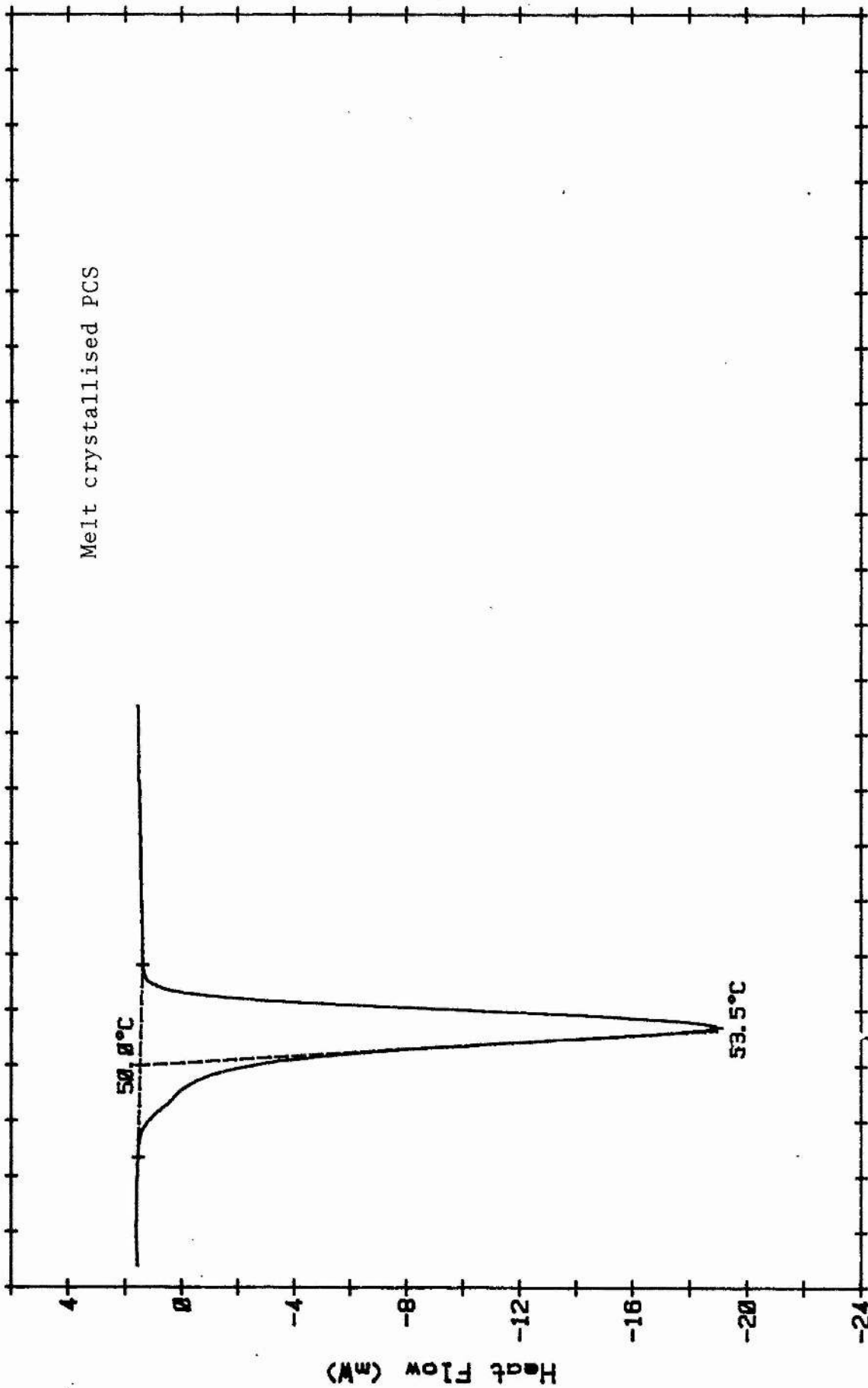

Conditioned PES
134. J/g
48.2°C
48.4°C
Heat Flow (mW)
4
0
-4
-8
-12
-16
-20
-24
30
40
50
60
70
80
90
100
110
120
130
140
Temperature (°C)
DuPont 1090


DSC
Melt crystallised PES
9.99 J/g
34.8°C
118. J/g
48.4°C
37.5°C
48.8°C
Heat Flow (mW)
Temperature (°C)
DuPont 1090

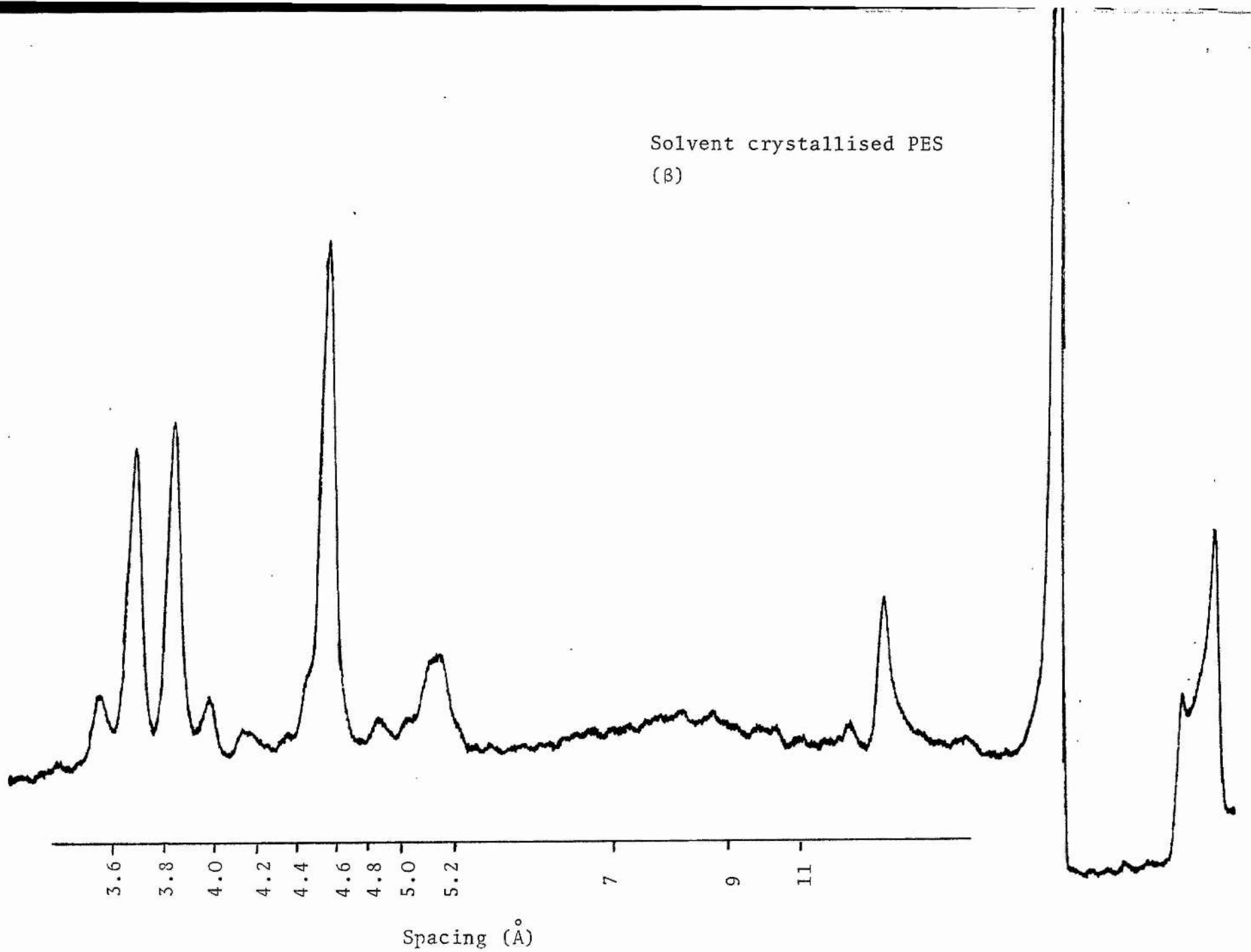
DSC

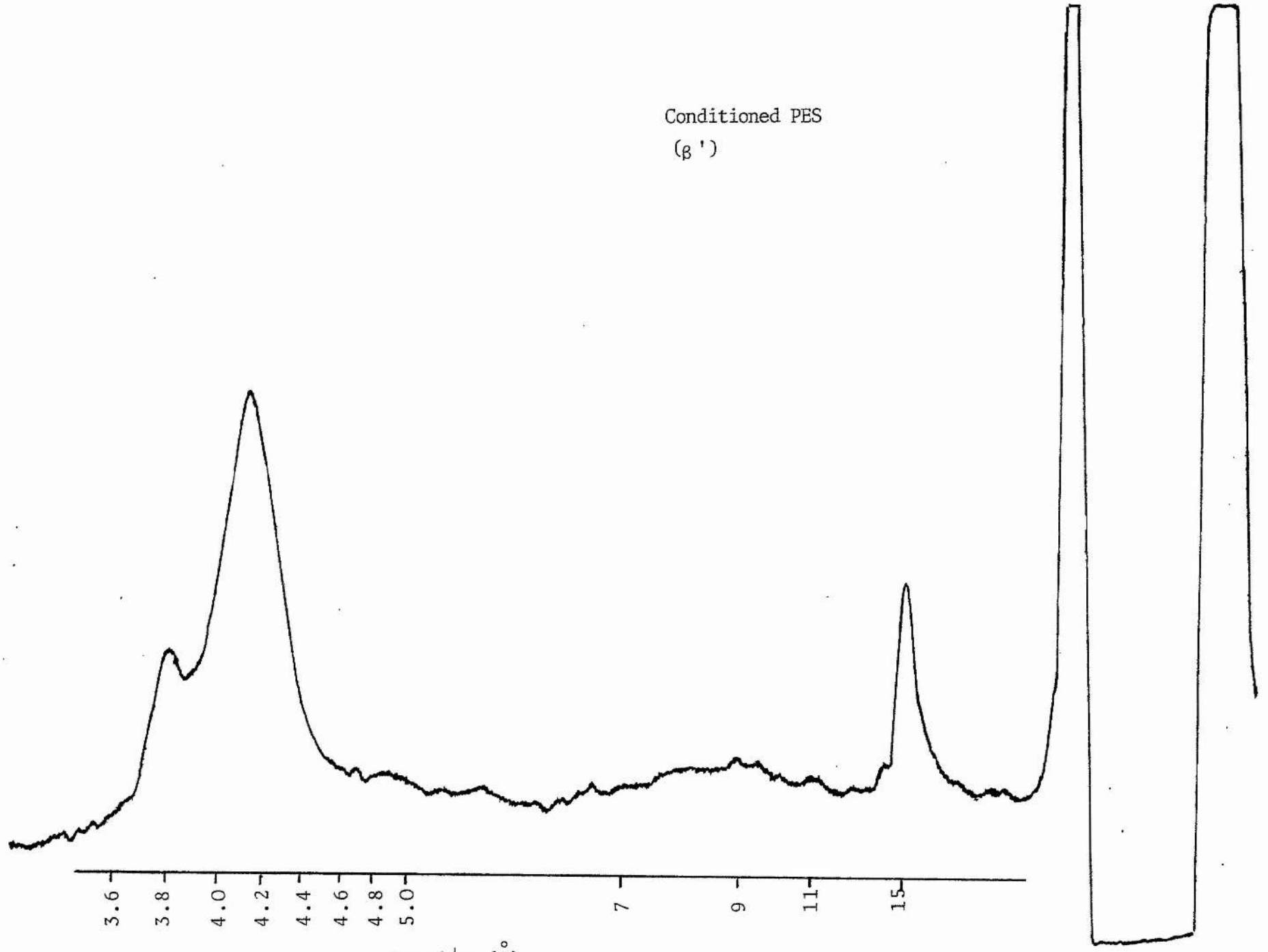

Solvent crystallised PCS
54.1°C
58.8°C
Heat Flow (mW)
4
0
-4
-8
-12
-16
-20
-24
30
40
50
60
70
80
90
100
110
120
130
140
Temperature (°C)
DuPont 1090

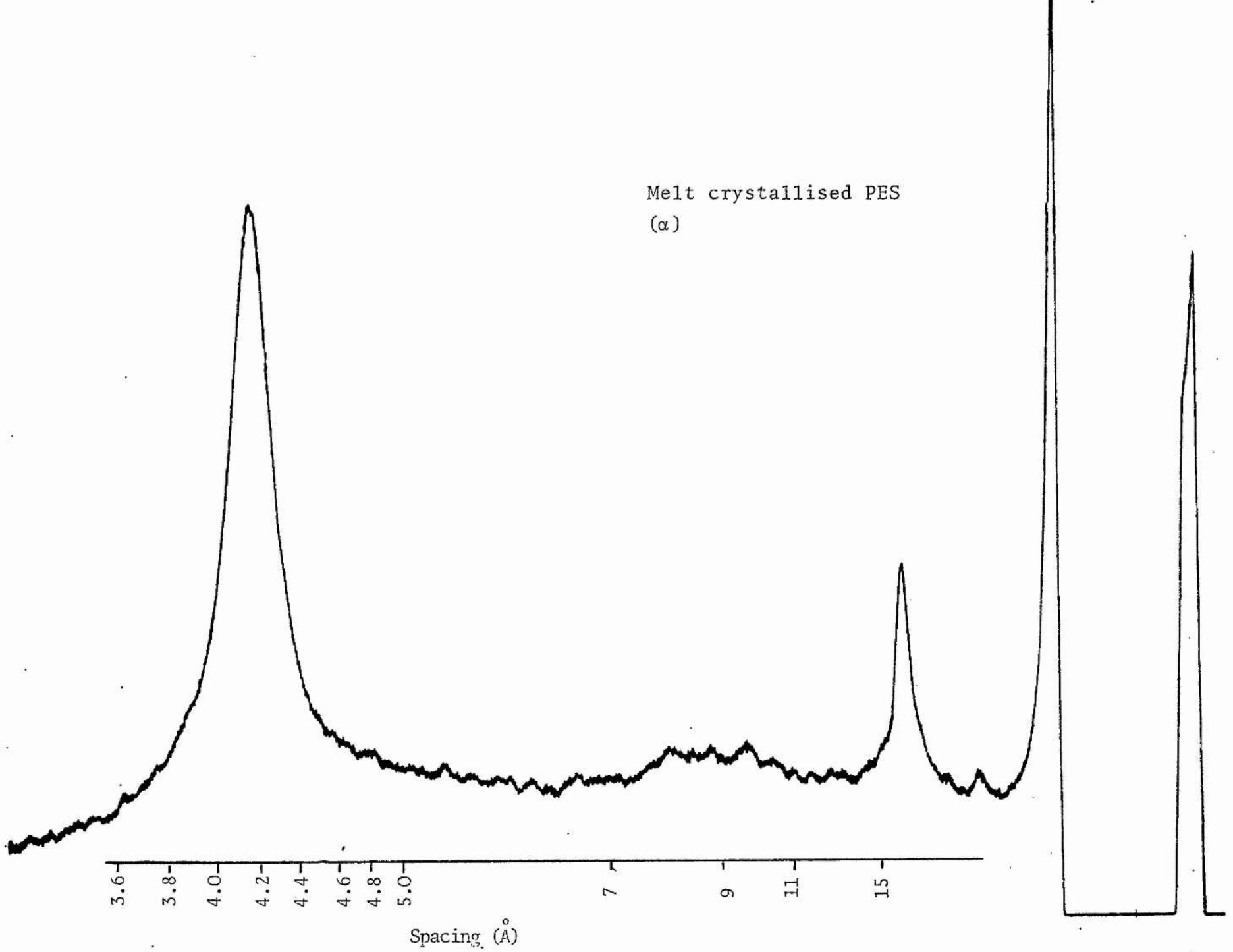
DSC

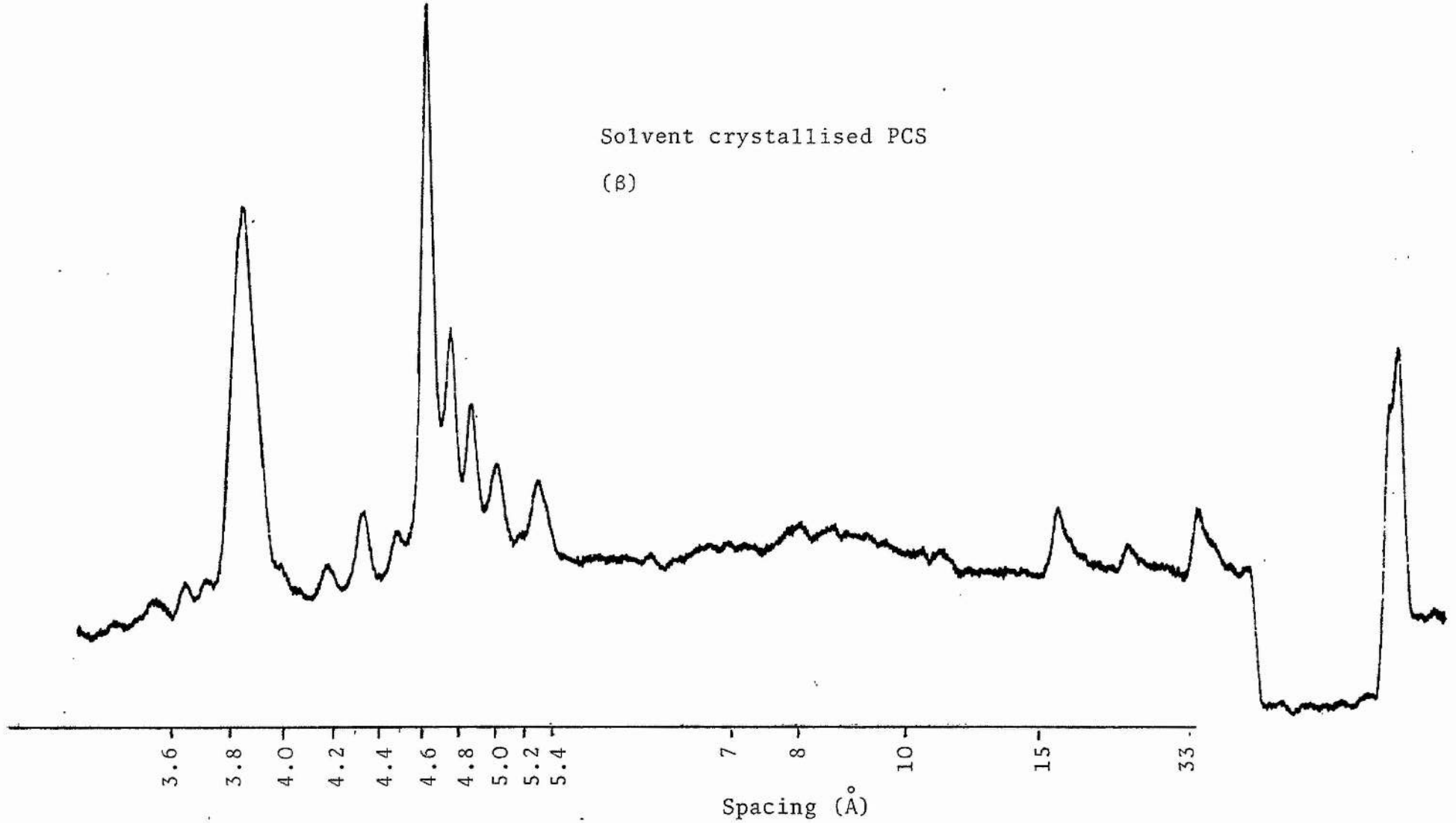


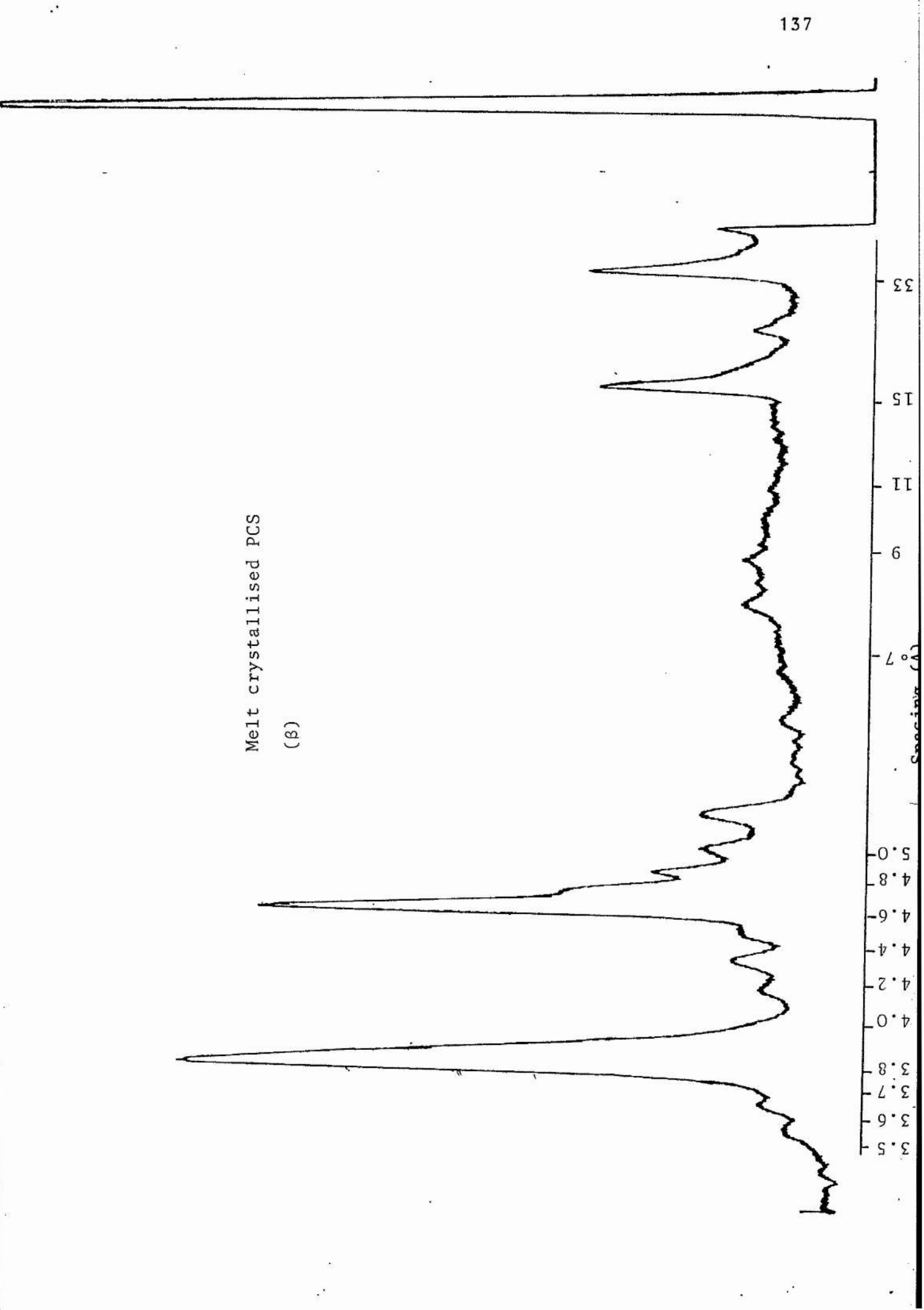
DuPont 1090


Solvent crystallised PES
(β)
3.6
3.8
4.0
4.2
4.4
4.6
4.8
5.0
5.2
7
9
11
Spacing (Å)


Conditioned PES
(β ')
3.6
3.8
4.0
4.2
4.4
4.6
4.8
5.0
7
9
11
15
Spacing (Å)


Melt crystallised PES
(α)
3.6
3.8
4.0
4.2
4.4
4.6
4.8
5.0
7
9
11
15
Spacing (Å)

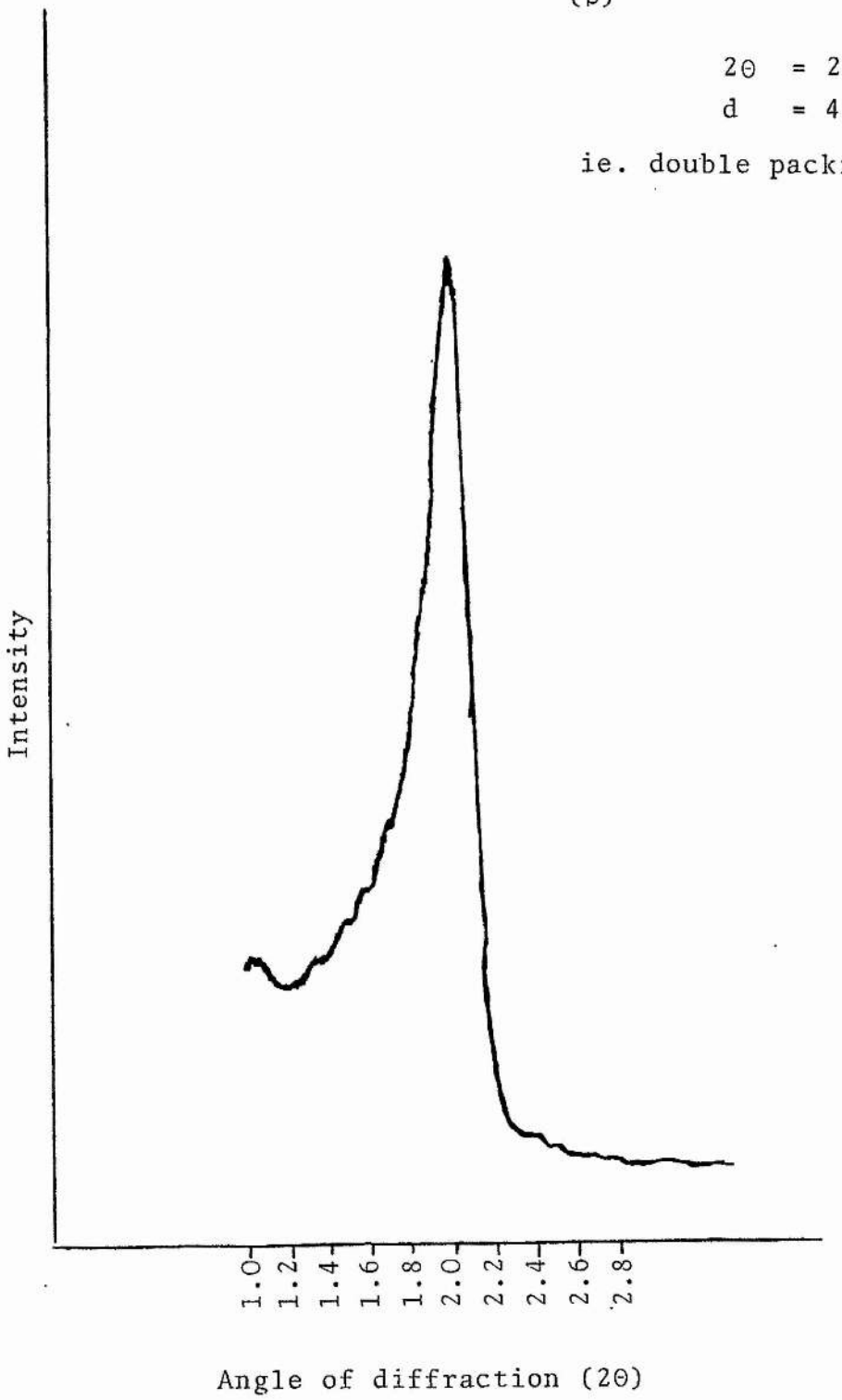

Solvent crystallised PCS
(β)
3.6
3.8
4.0
4.2
4.4
4.6
4.8
5.0
5.2
5.4
7
8
10
15
33
Spacing (Å)


Melt crystallised PCS
(β)
33
15
11
9
7
Spacing (Å)
5.0
4.8
4.6
4.4
4.2
4.0
3.8
3.7
3.6
3.5

Solvent crystallised PES
(β)

$2\theta = 2.0$
$d = 44.2$ Å

ie. double packing



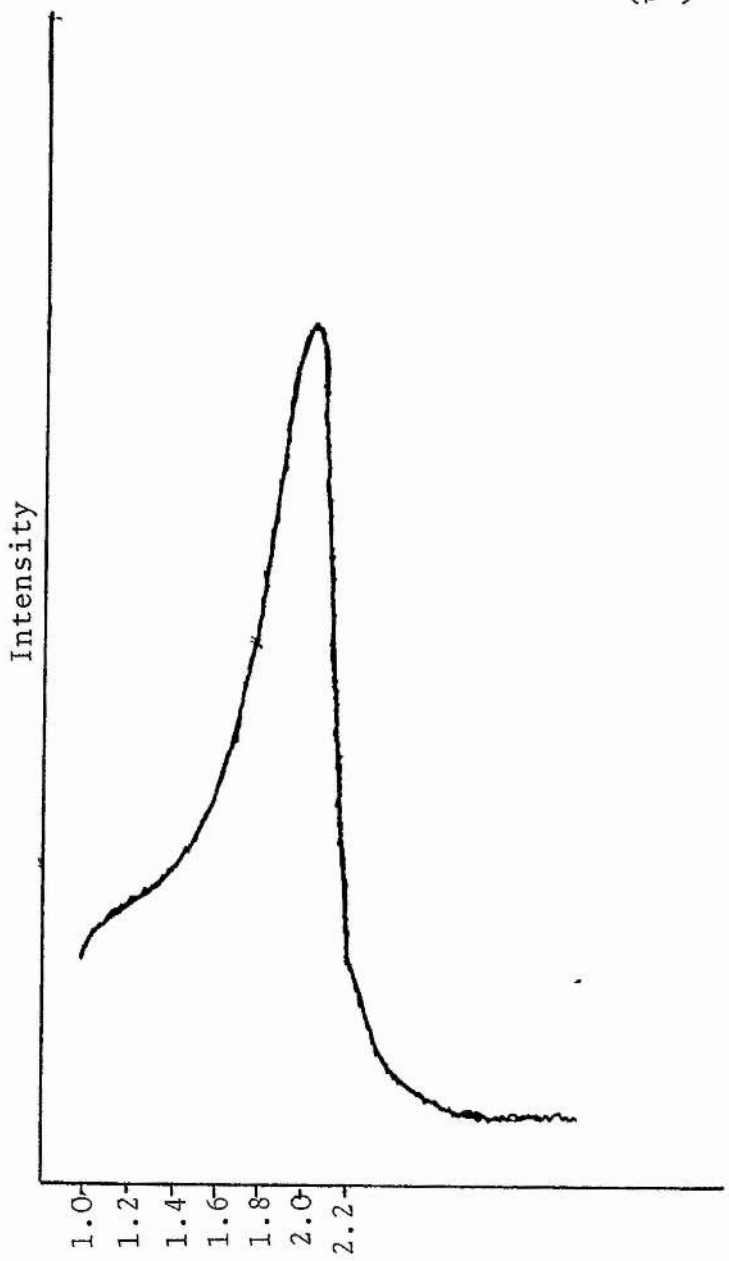
Conditioned PES

(β')

2θ = 2.0

d = 44.4 Å

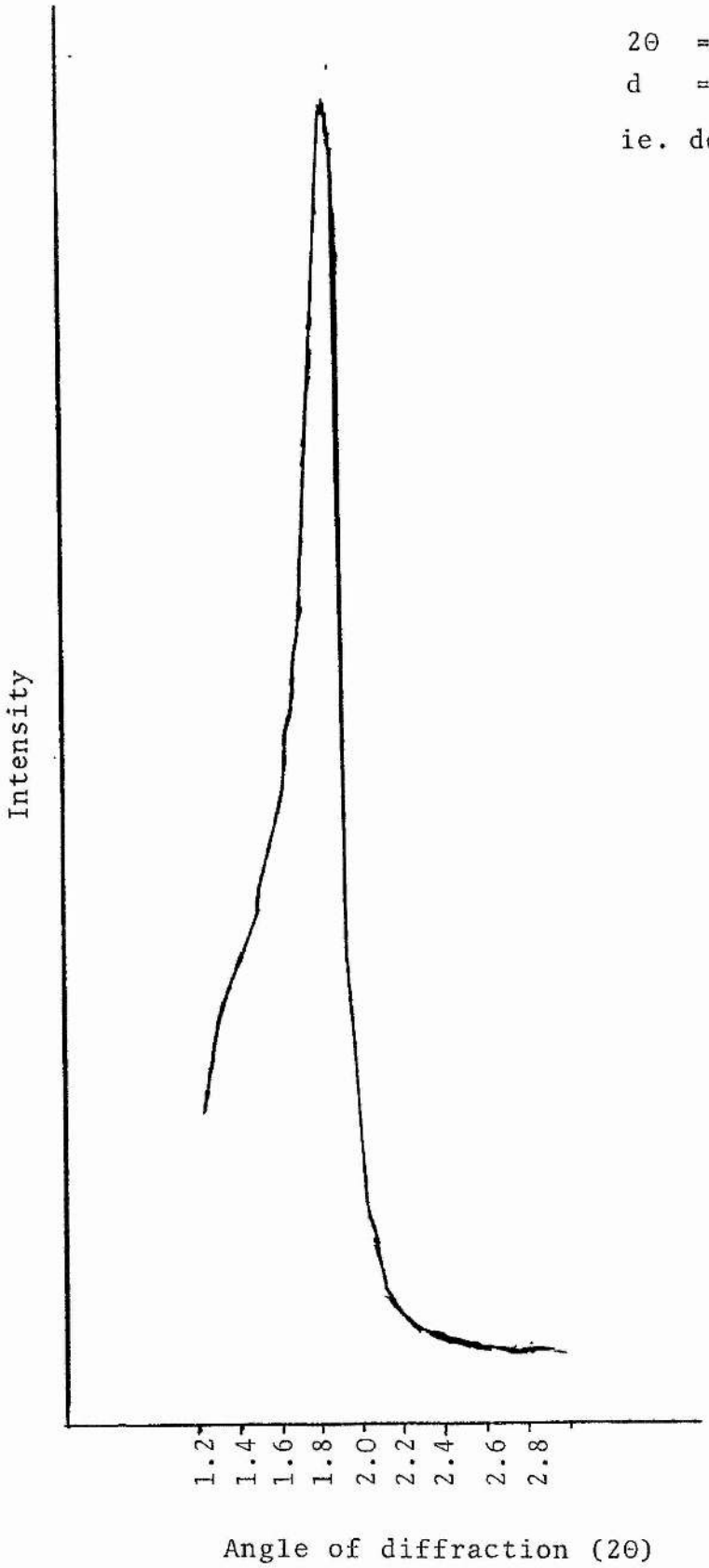
ie. double packing

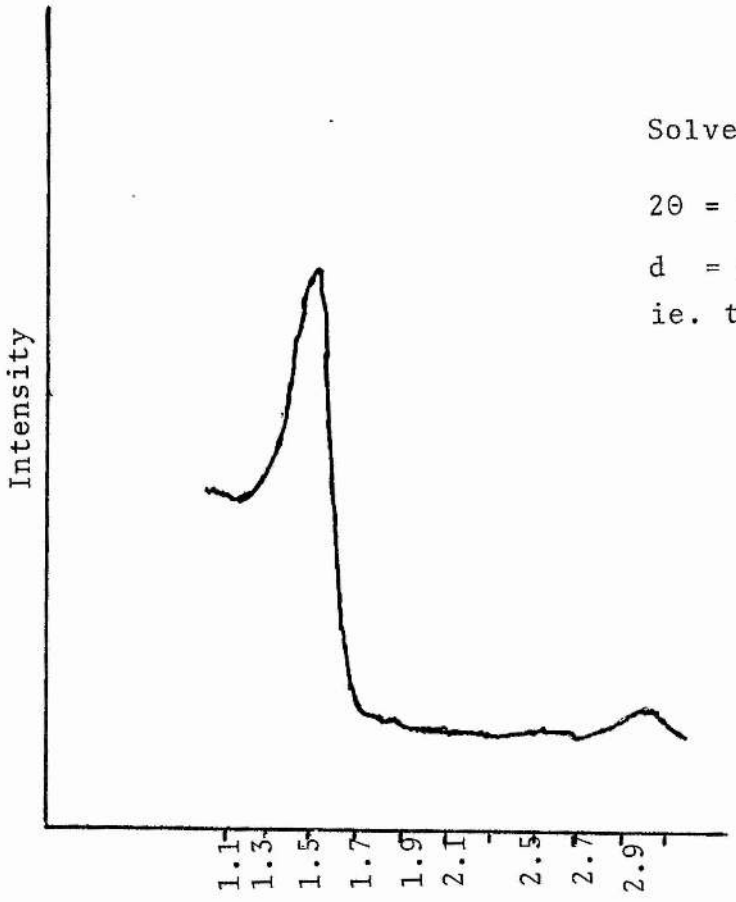


Melt crystallised PES
(α)

$2\theta = 1.8$
$d = 47.8$ Å

ie. double packing



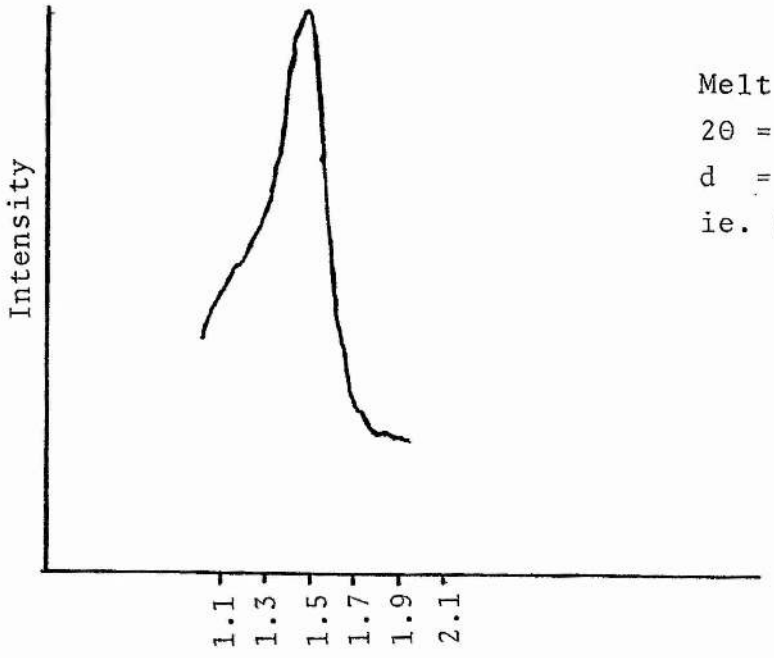


Solvent crystallised PCS

2θ = 1.5

d = 58.9 Å

ie. triple packing.



Melt crystallised PCS

2θ = 1.45

d = 60.9 Å

ie. triple packing.

A P P E N D I X 4

Some literature values of long spacings and melting points of β-2 phase triglycerides

De Jong and Van Soest, *Acta Cryst.*, 1978, B34, 1573.

Triglyceride	Long spacing	Melting point
SSS	52.9[1],51.4[2],49.8[1],48.9[2], 44.7[3],40.7[4],40.5[1],39.6[2], 36.0[1],35.0[2].	73.5[6],72.6[3], 72.5[5].
LLL	38.0[1],34.6[8],31.4[7],28.3[1], 25.1[1].	46.5[6],46.4[7,9].
MMM	42.9[1],40.4[1],35.8[9],35.45[10], 32.0[1],28.7[1]	58.5[6],57.0[9].
AAA	49.5[6]	78.1[6]
PEE	44.1[11]	40.3[11]
SMM	39.5[12]	46.0[12]

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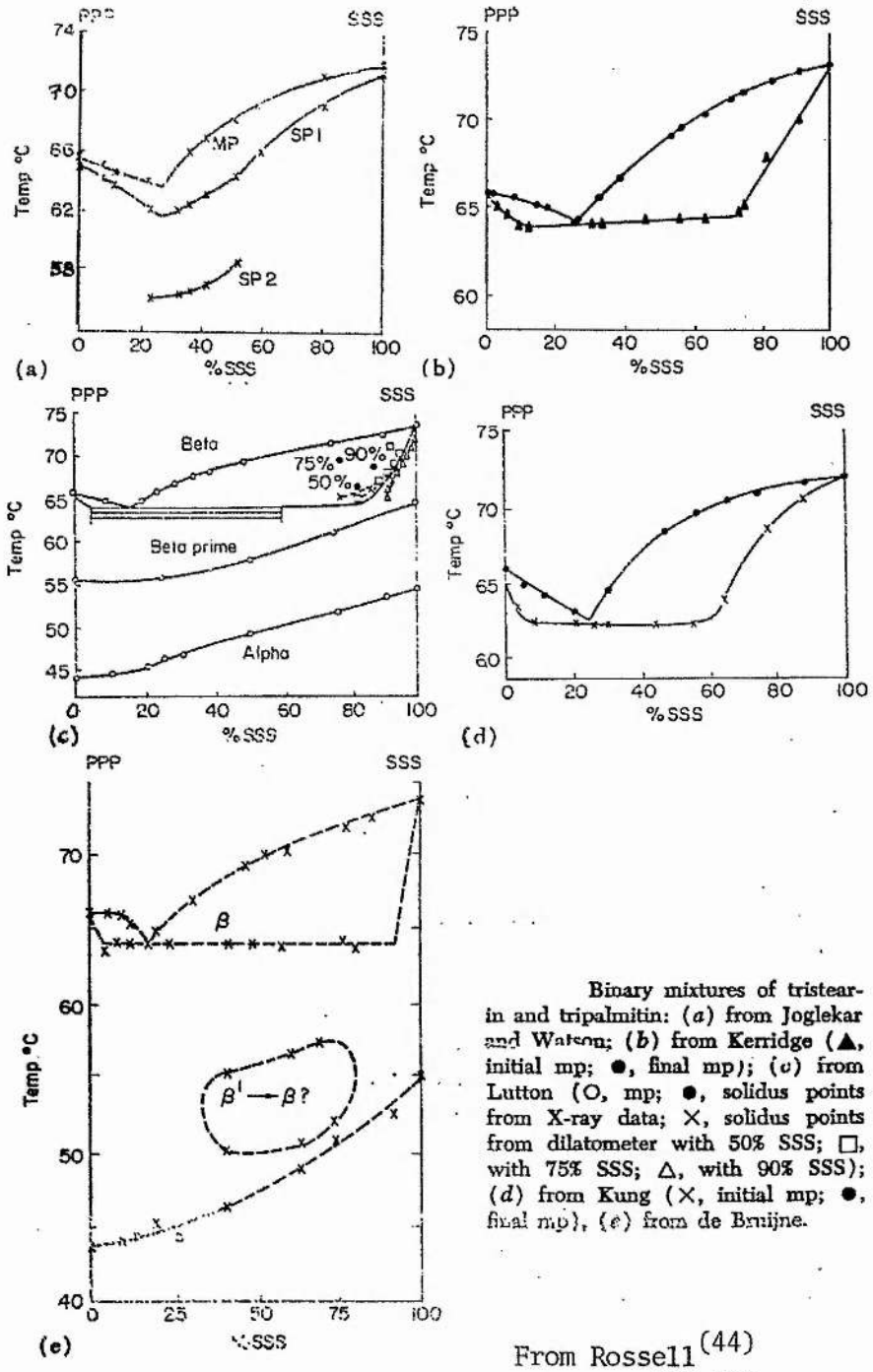
APPENDIX 5



Binary mixtures of tristearin and tripalmitin: (*a*) from Joglekar and Watson; (*b*) from Kerridge (▲, initial mp; ●, final mp); (*c*) from Lutton (O, mp; ●, solidus points from X-ray data; ×, solidus points from dilatometer with 50% SSS; □, with 75% SSS; △, with 90% SSS); (*d*) from Kung (×, initial mp; ●, final mp), (*e*) from de Bruijne.

From Rossell[44]
Diags a-c[56-59]

A P P E N D I X 6

X-ray Powder Diffraction Equipment

1. Specifications

1.1 X-ray Generator

Make: Philips

Model No. PW1011/00

High tension voltage range	10 - 54 kV
Tube current range	6 - 36 mA
Maximum permissible load	1600 W
Maximum power consumption	2900 W

Maximum current taken from mains 27A at 220V

Cooling water consumption 3.5 L (6.2 pints) per minute

Required water pressure 2.5 kg/cm² (35 lbs/in²)

Maximum peak water pressure 5 kg/cm² (70 lbs/in²)

1.2 X-ray Diffraction Tube

Make: Philips

Type: RDF 50/1 (fine focus)

Anode material	Copper
Maximum rating	1200 W 50 kV
Wavelength of Kα radiation	1.5418 Å
Projected focal spot size for 6° take off angle	Point focus 0.4 x 0.8 mm Line focus 0.04 x 8 mm

1.3 X-ray Film

Make: Kodak

Type: DEF-59 Direct Exposure Film

Camera: DPT

Exposure time: 15 mins.

1.4 Recording Microdensitometer

Make: Joyce-Loebl and Co. Ltd, Gateshead

Model: III CS.

1.5 Geiger Counter

Make: Rigakic Denki

Type: 5705

1.6 Ratemeter

Make: Panak Equipment Ltd

Type: P7900 A

Settings: EHT - 1300 V

Disc Bias - 30 mV

Scintillation Gain - x1

Time Constant - 30s

2. Calculation of "d-Spacings"

The distances between the planes of molecules within a crystal lattice are known as the "d-Spacings". Since they are characteristic of any particular compound or of polymorphic forms of the same compound they can be used for identification purposes.

The Bragg equation governs the reflection (diffraction) of X-rays from crystals.

$$n\lambda = 2d \sin \theta$$

where

n = 1,2,3,4,5 etc.

λ = wavelength of X-rays

d = "d-Spacing"

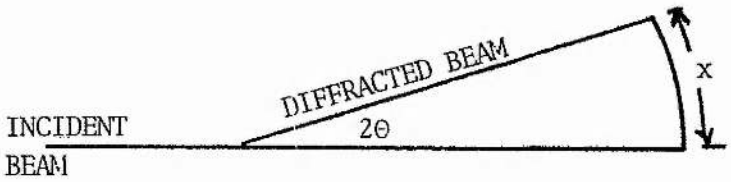
2θ = the angle between the diffracted and incident beams.

The calculation of the "d-Spacing" involves three stages:

1. Calculation of 2θ from the distances between the diffraction patterns on the X-ray film and the geometry of the camera.
2. Knowing the value of 2θ then the value of Sin θ can be calculated.
3. Knowing the value of Sin θ and λ the wavelength of the X-rays then from the Bragg equation the value of "d" can be calculated.

2.1 DPT Camera

This camera has a curved film holder the radius of which is equal to the sample to film distance.



$$2\theta = \frac{x}{SFD} \text{ radians} = \frac{57.3x}{SFD} \text{ degrees}$$

where x = distance on film (cm) of diffraction line from that of the undeviated beam.

SFD = sample to film distance (cm).

For DPT Camera SFD = 9.8 cm 2θ = 5.847x.

The d-Spacing can be calculated from 2θ using Bragg equation in the following form:

$$d = \frac{n\lambda}{2 \sin \theta} = \frac{1.5418}{2 \sin \theta}$$ for Cu_k radiation $\lambda = 1.5418°A$

Normally the diffraction pattern on the film is converted into a microdensitometer trace using the Joyce Loebl microdensitometer. If the normal arm ratio of 5:1 is used then the distance on the trace of a diffracted line from the undeviated beam will be 5 times the corresponding distance on the film. Allowance for this must be made in the above calculations of 2θ from x.

2.2 Rigaku Denki Camera

This can be used with film or with a geiger counter which scans across the diffracted beam. For the purposes of this project, the counter method was the technique used.

Counter Method

Using this method the geiger counter scans through the diffraction angle and detects the change in the intensity of the diffracted beam. The signal from the counter is converted by a ratemeter to an analogue output which can be recorded on a chart recorder. During the scan a series of peaks are recorded on the chart which correspond to the diffraction lines obtained by the film method. The diffraction angle (2θ) corresponding to a particular diffraction line can be calculated from the distance of the peak from the start of the trace. Knowing the value of 2θ then $\sin\theta$ can be calculated and using the Bragg equation the d-Spacing can be calculated as described in Section 2.